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Alcaraz Asensio et al.

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(54) **BLADDER CANCER DIAGNOSIS AND/OR PROGNOSIS METHOD**

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(58) **Field of Classification Search**
USPC 435/6.1
See application file for complete search history.

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(57) **ABSTRACT**

A high sensitive and specific non-invasive bladder cancer diagnosis and/or prognosis method based on the detection and quantification of the gene expression of a combination of bladder tumor markers in bladder fluids is provided. A preferred combination of the markers consists of the combination of ANXA10, C14orf78, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6, TERT, ASAM and MCM10 genes.

4 Claims, 43 Drawing Sheets

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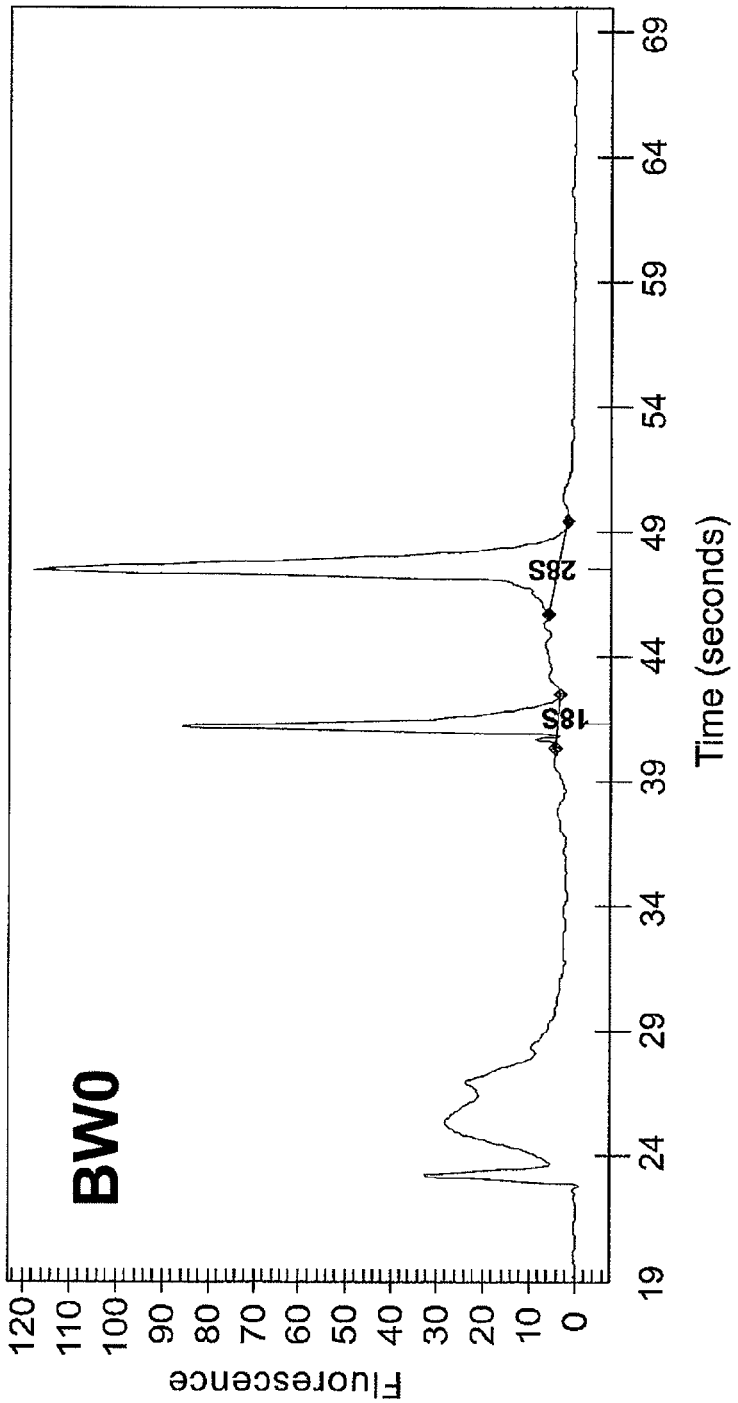
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28S
18S
Fluorescence
Time (seconds)
0
10
20
30
40
50
60
70
80
90
100
110
120
19
24
29
34
39
44
49
54
59
64
69

FIG. 1A

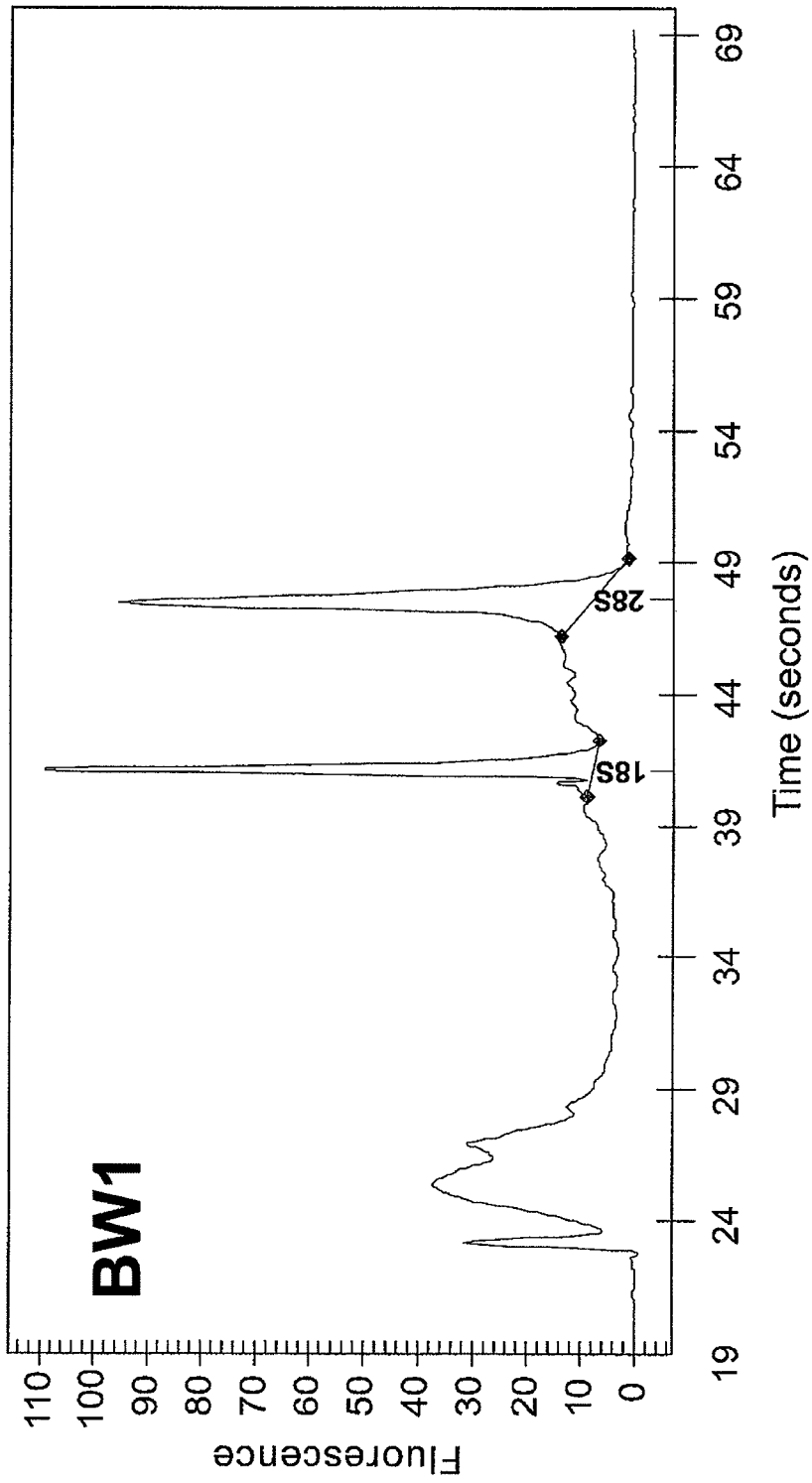

BW1
Fluorescence
110
100
90
80
70
60
50
40
30
20
10
0
19
24
29
34
39
44
49
54
59
64
69
Time (seconds)
18S
28S

FIG. 1B

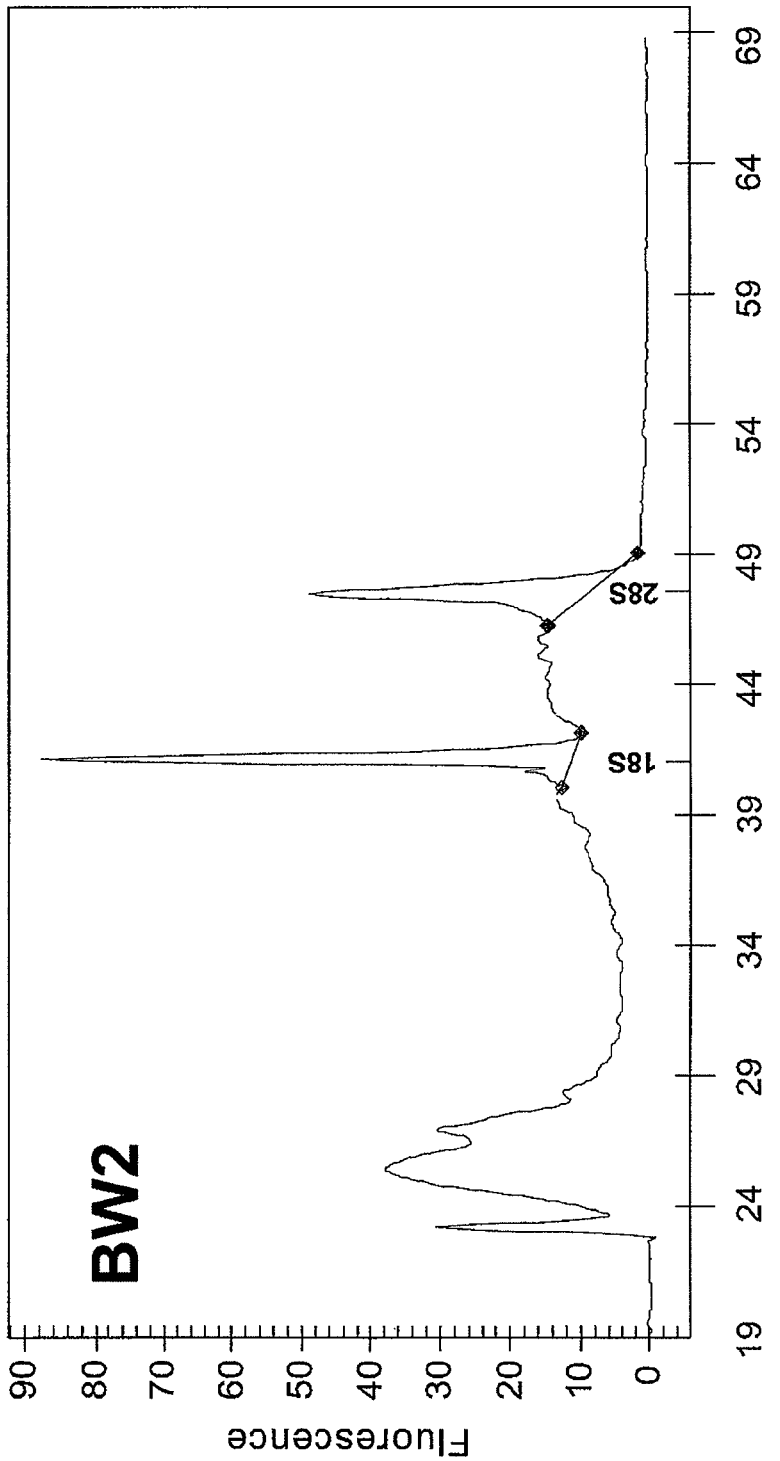

BW2
Fluorescence
0
10
20
30
40
50
60
70
80
90
Time (seconds)
19
24
29
34
39
44
49
54
59
64
69
18S
28S

FIG. 1C

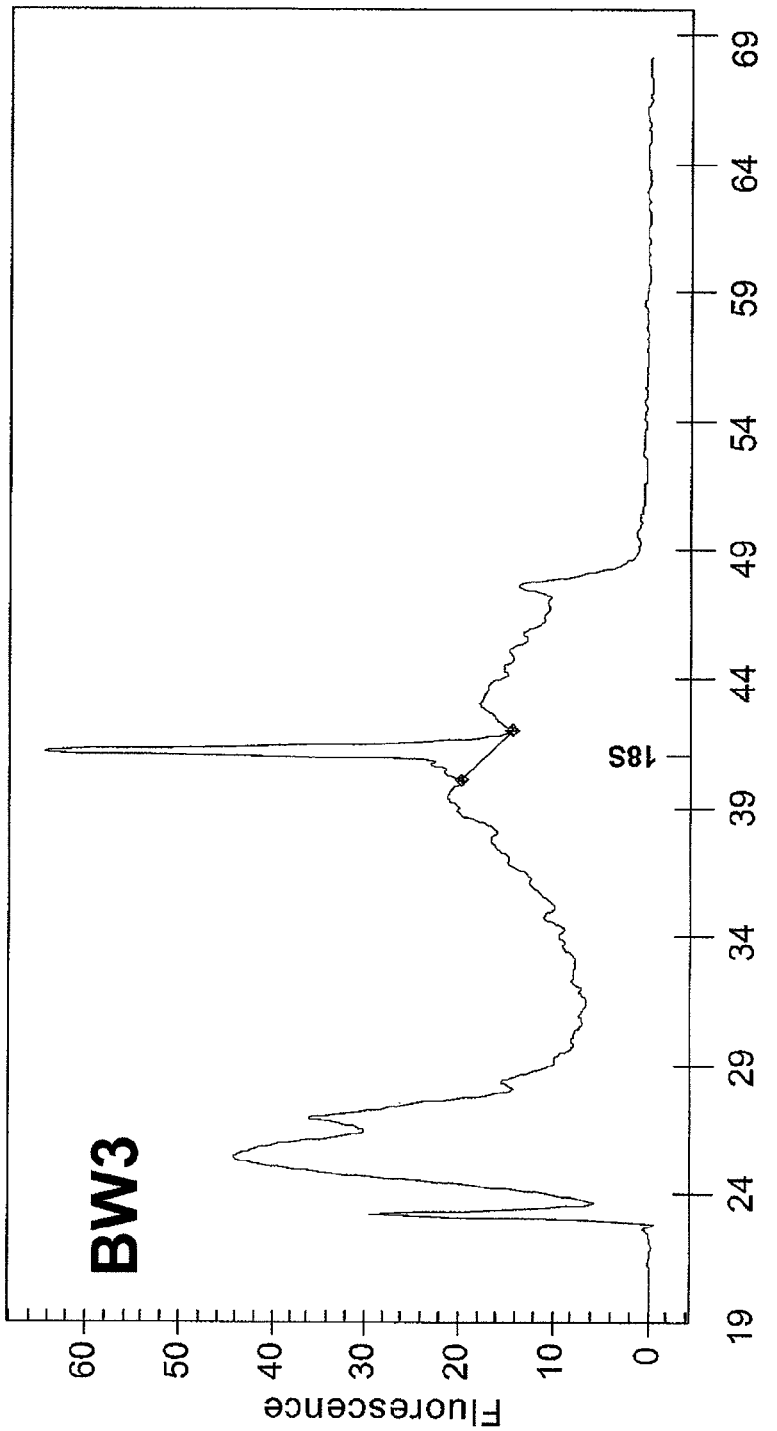

BW3
18S
Fluorescence
0
10
20
30
40
50
60
Time (seconds)
19
24
29
34
39
44
49
54
59
64
69

FIG. 1D

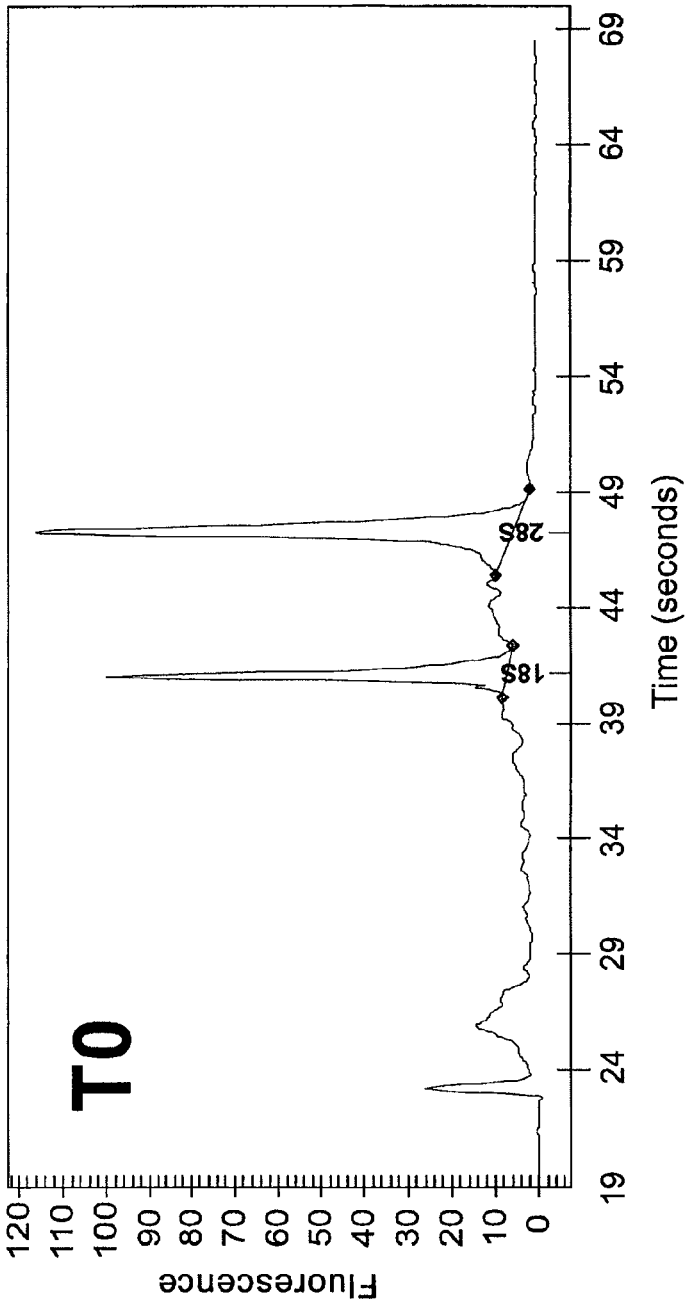

T0
28S
18S
Fluorescence
120
110
100
90
80
70
60
50
40
30
20
10
0
19
24
29
34
39
44
49
54
59
64
69
Time (seconds)

FIG. 1E

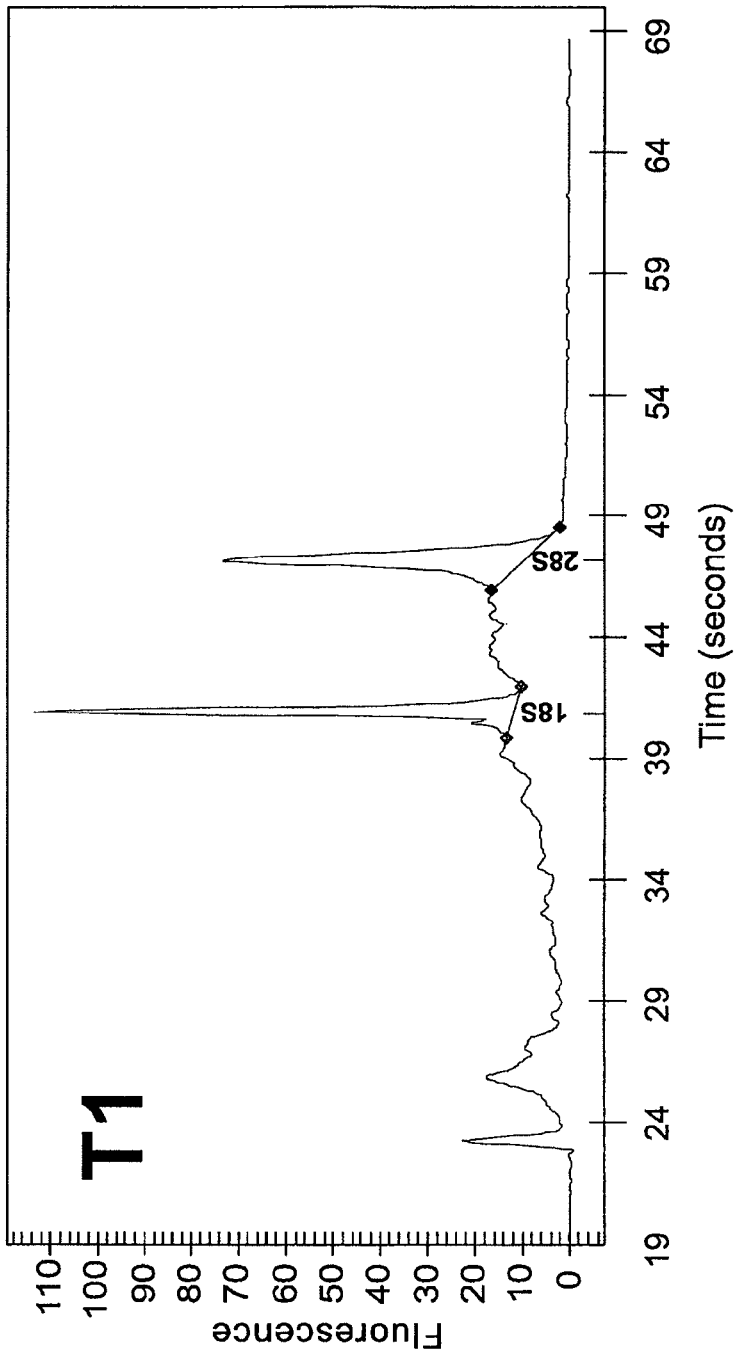

T1
18S
28S
Fluorescence
0
10
20
30
40
50
60
70
80
90
100
110
Time (seconds)
19
24
29
34
39
44
49
54
59
64
69

FIG. 1F

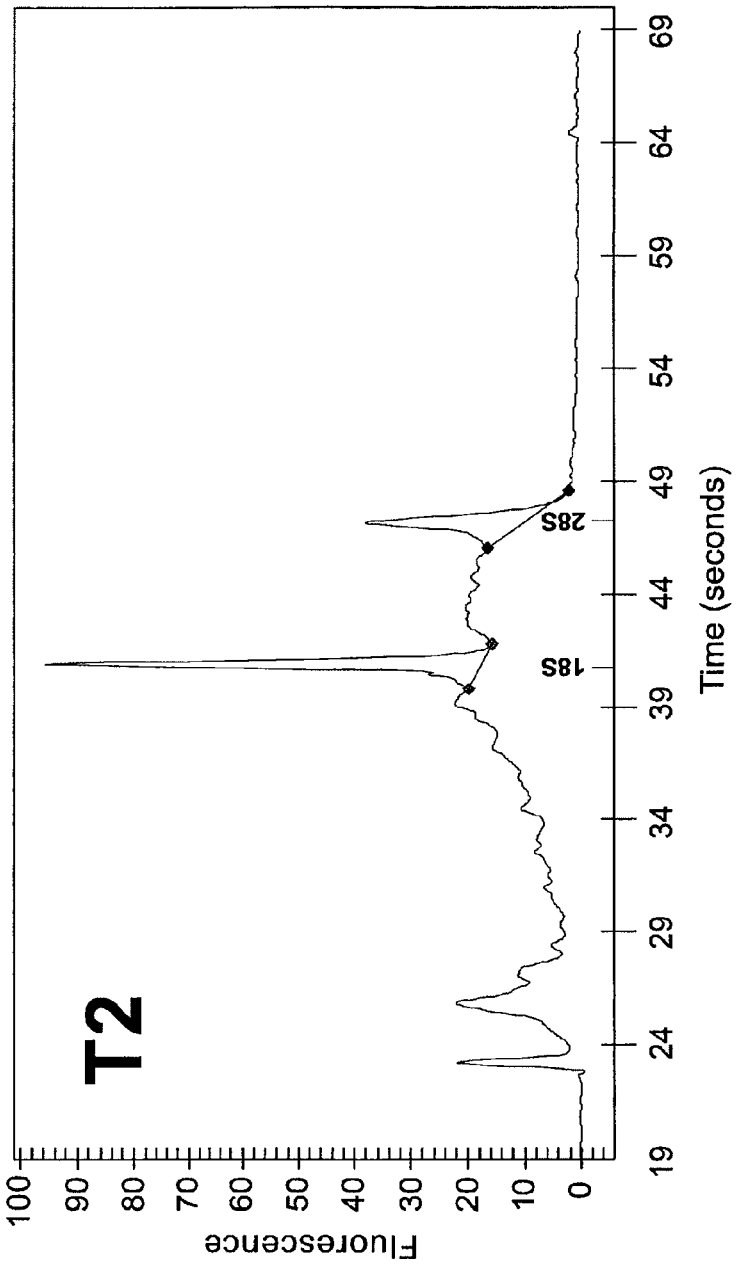

T2
Fluorescence
100
90
80
70
60
50
40
30
20
10
0
Time (seconds)
19
24
29
34
39
44
49
54
59
64
69
18S
28S

FIG. 1G

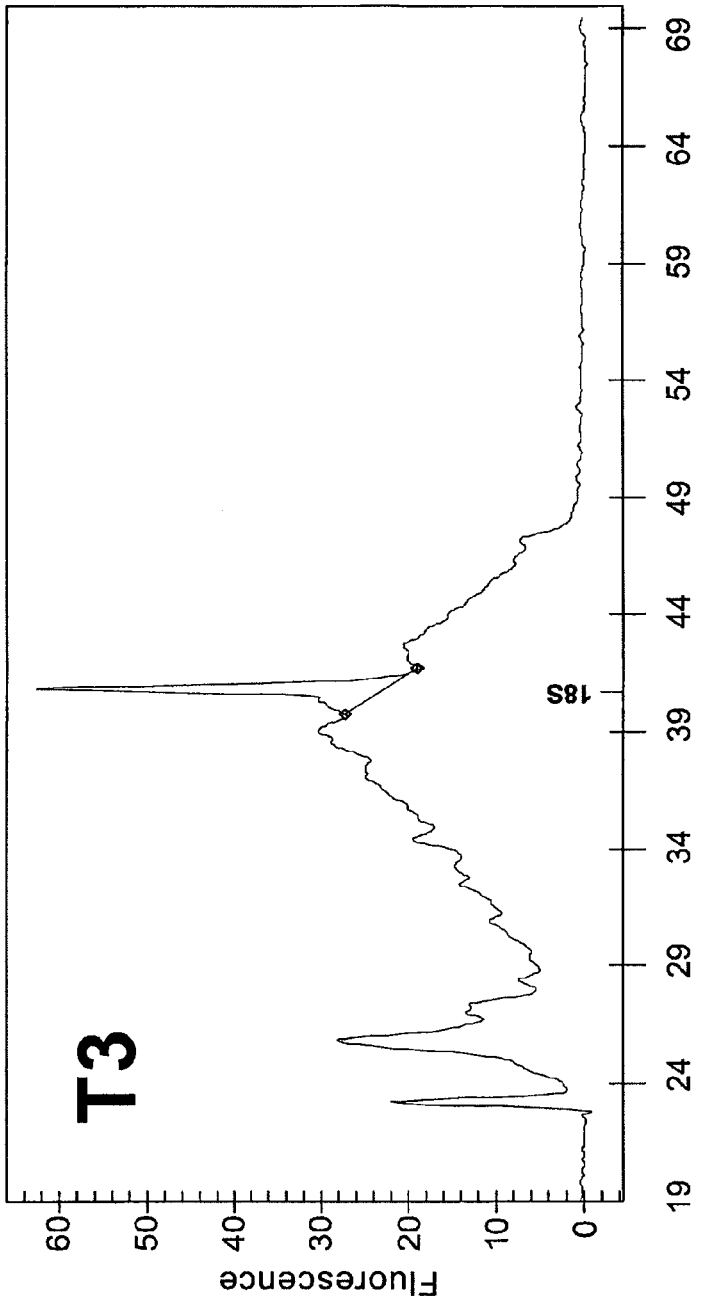

T3
18S
Fluorescence
0
10
20
30
40
50
60
Time (seconds)
19
24
29
34
39
44
49
54
59
64
69

FIG. 1H

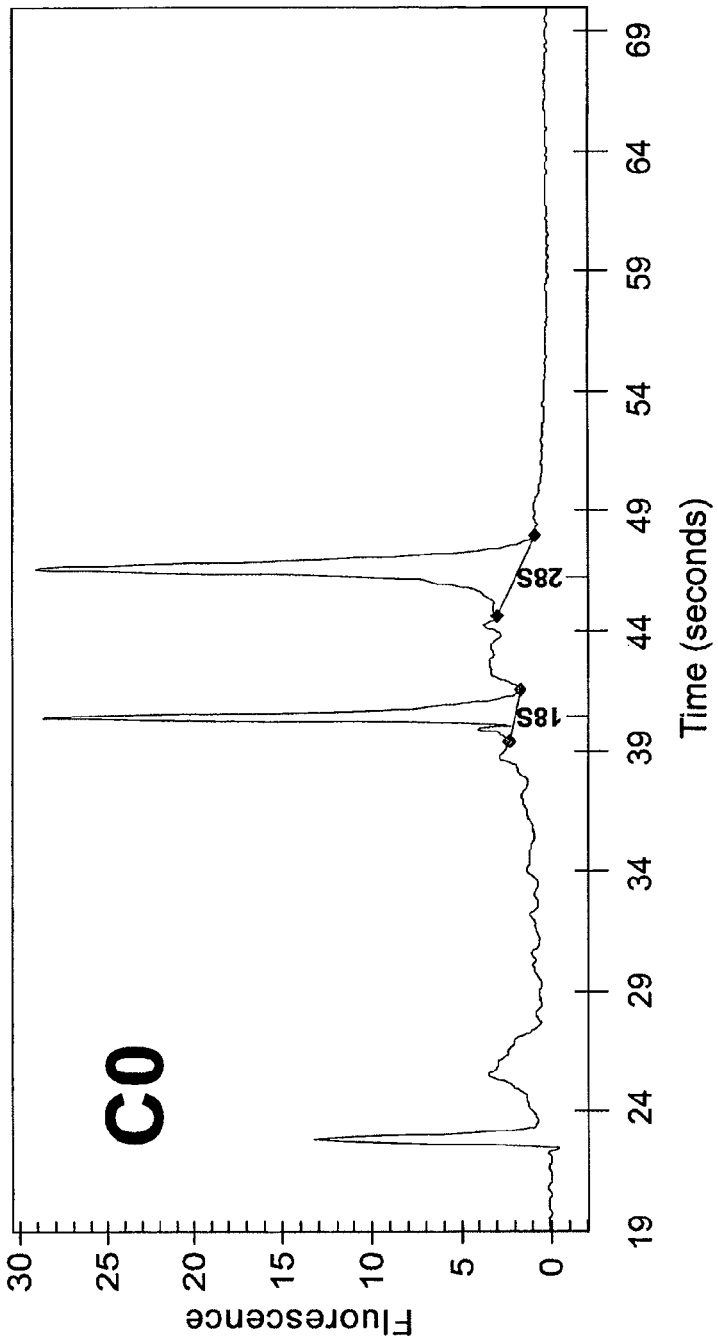

C0
18S
28S
Fluorescence
Time (seconds)
0
5
10
15
20
25
30
19
24
29
34
39
44
49
54
59
64
69

FIG. 11

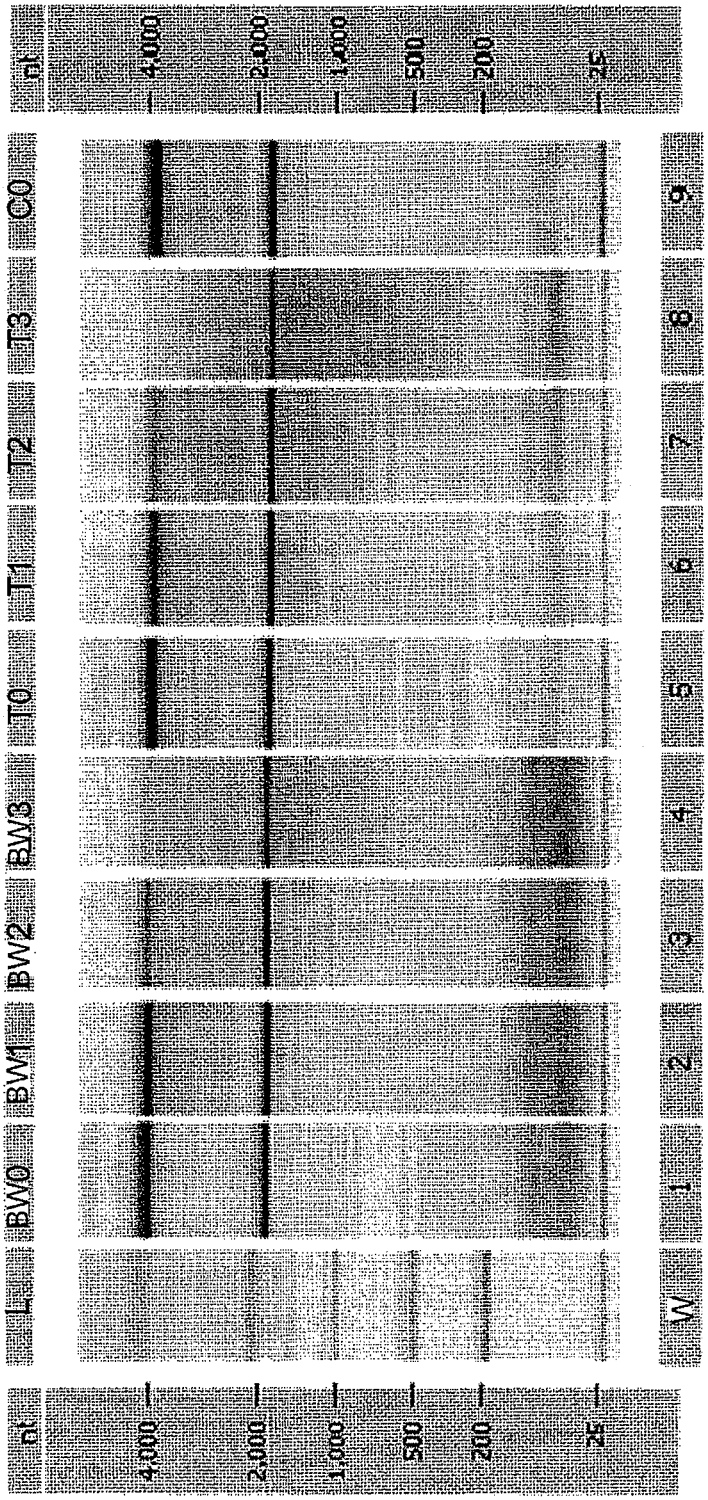

nt
4,000
2,000
1,000
500
200
25
L
BW0
BW1
BW2
BW3
T0
T1
T2
T3
C0
1
2
3
4
5
6
7
8
9
W

FIG. 1J

a)

	T0	T1	T2	T3	BW0	BW1	BW2
T1	[illegible]						
T2	[illegible]	[illegible]					
T3	[illegible]	[illegible]	[illegible]				
BW0	59	59	56	54			
BW1	60	59	56	54	[illegible]		
BW2	58	56	53	52	[illegible]	[illegible]	
BW3	56	56	53	52	[illegible]	[illegible]	[illegible]

b)

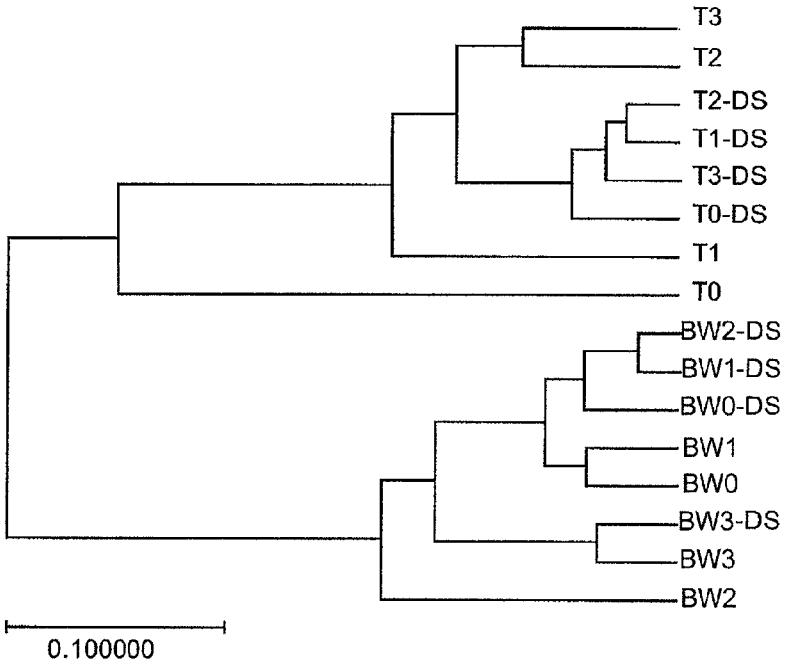


FIG. 2

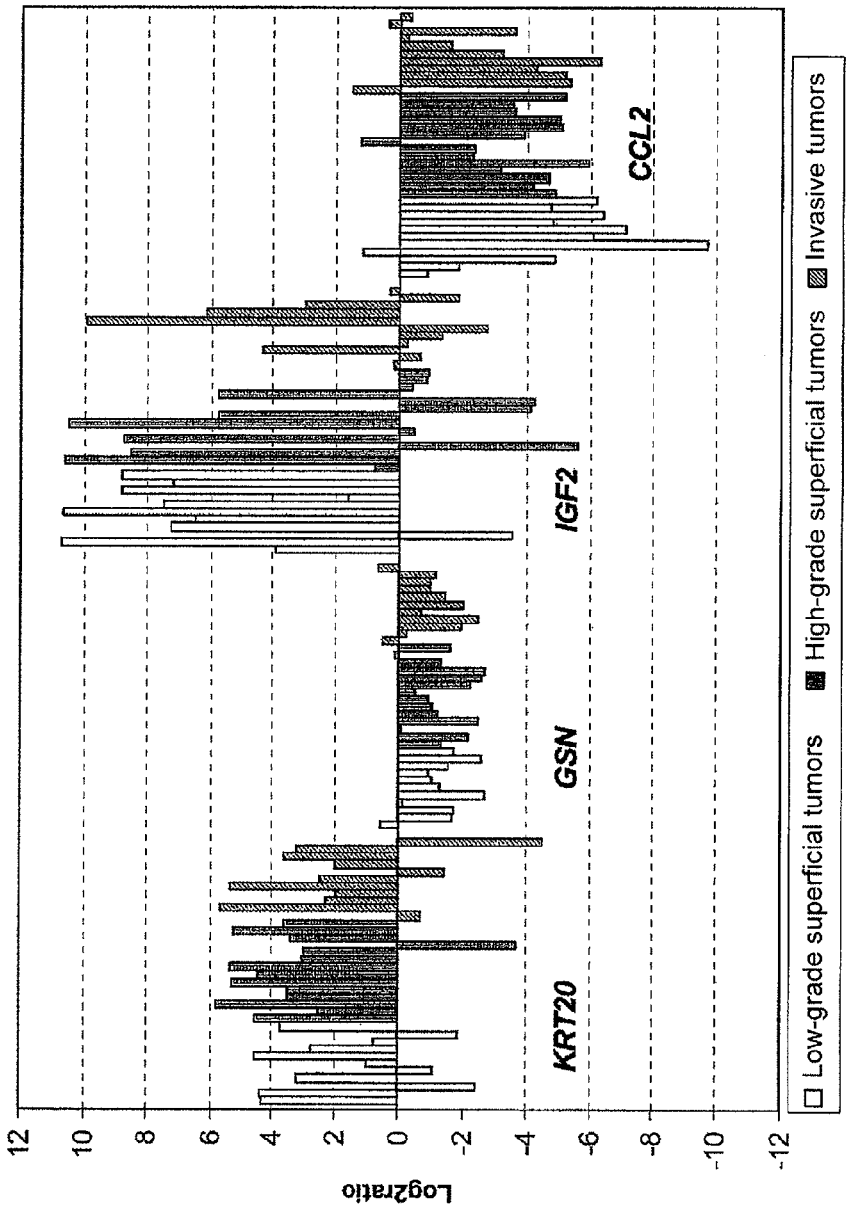

Log2ratio
12
10
8
6
4
2
0
-2
-4
-6
-8
-10
-12
CCL2
IGF2
GSN
KRT20
Low-grade superficial tumors
High-grade superficial tumors
Invasive tumors

FIG. 3

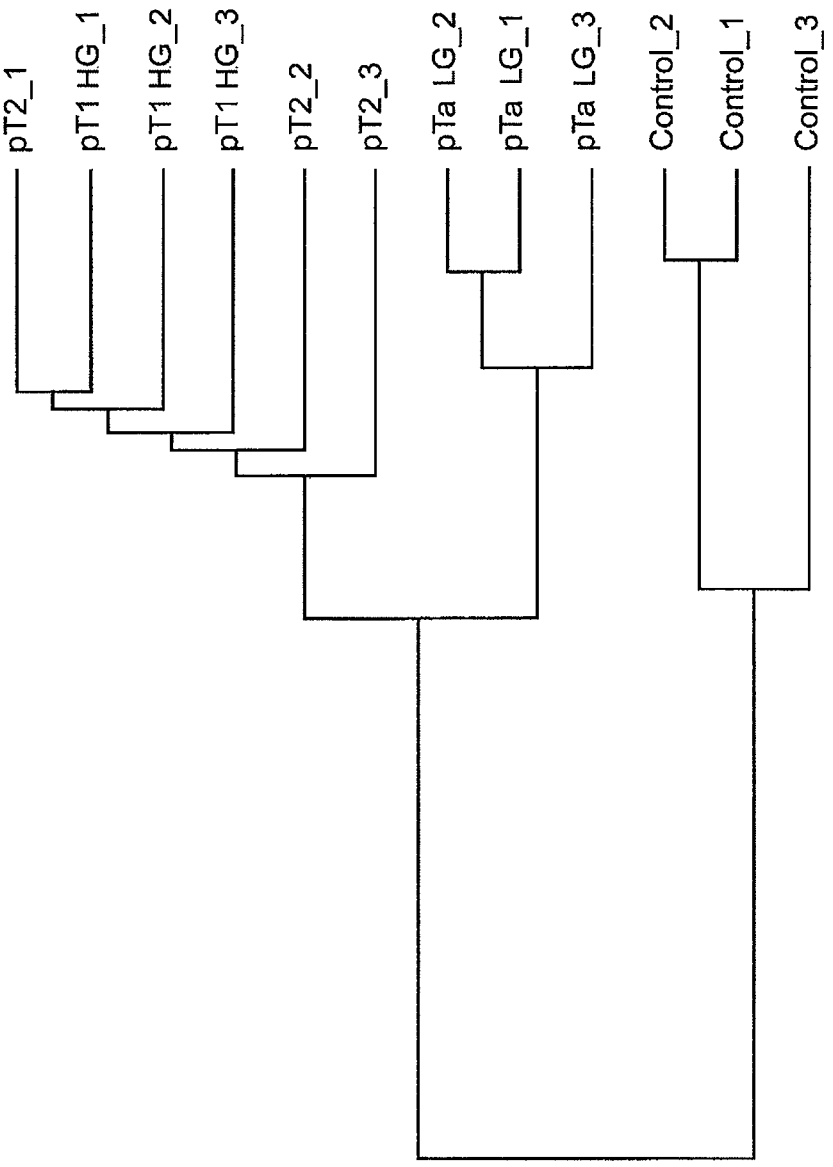
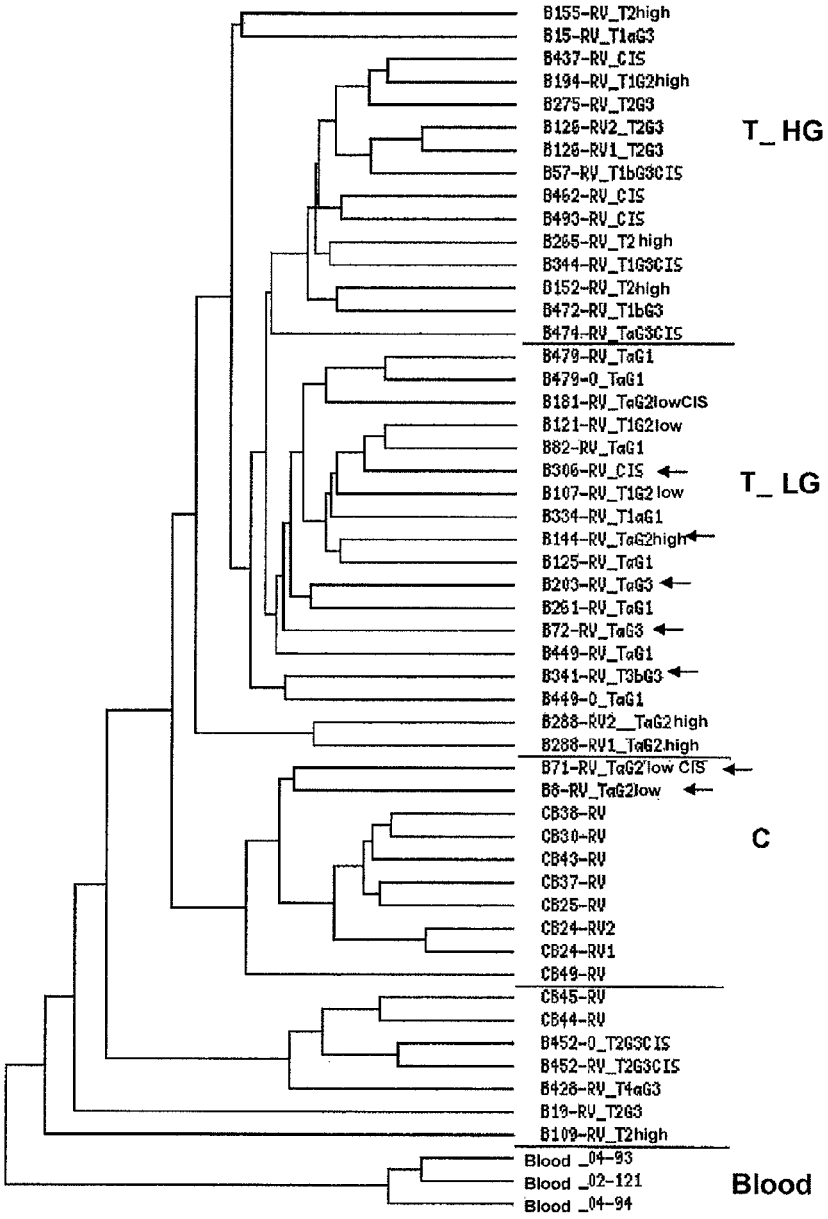

pT2_1
pT1 HG_1
pT1 HG_2
pT1 HG_3
pT2_2
pT2_3
pTa LG_2
pTa LG_1
pTa LG_3
Control_2
Control_1
Control_3

FIG. 4

	Pools			Individual samples					
	No	µarrays	qRT-PCR	Mean	1	2	3	4	5
TCN1	1	6,55	8,82	8,01	1,23	-0,29	6,99	8,12	9,78
	2	6,71	9,42	8,15	6,80	7,47	-0,59	3,97	10,12
	3	5,10	8,34	6,48	7,50	6,73	5,89	5,28	5,93
SORBS1	1	-5,47	-4,10	-5,24	-8,44	-7,04	-4,82	-6,42	-3,75
	2	-3,88	-8,08	-3,19	-6,10	-1,30	-7,30	-6,45	---
	3	-5,60	-4,09	-5,21	-6,42	-6,59	-3,75	-6,88	---
MYH11	1	-6,03	-7,60	-6,70	-11,53	-9,47	-6,03	-9,57	-5,06
	2	-3,52	-4,40	-4,11	-6,89	-2,19	-9,32	-8,71	---
	3	-6,93	-7,69	-8,36	-7,99	-9,95	-7,59	-8,91	---
SRPX	1	-4,97	-6,36	-5,45	-12,15	-8,56	-3,93	-6,09	-5,01
	2	-5,59	-5,02	-5,58	-5,60	-4,26	-8,56	-6,95	---
	3	-5,90	-6,45	-6,46	-7,01	-8,13	-5,03	-8,04	[illegible]
CRH	1	3,53	5,44	3,68	-5,82	0,11	-2,12	5,36	4,44
	2	7,25	10,56	7,95	3,18	8,67	9,14	2,64	---
	3	6,11	8,22	5,95	7,83	4,29	-2,12	-1,71	---
KRT14	1	7,53	8,15	7,46	1,49	0,77	1,09	1,47	0,27
	2	3,89	3,68	2,78	4,04	3,24	1,26	1,07	2,91
	3	2,55	2,25	1,82	3,37	2,70	2,98	2,17	1,18
RRM2	1	5,47	4,97	3,81	1,83	4,27	3,16	4,91	3,09
	2	6,02	5,41	4,38	4,04	4,95	3,16	3,65	5,13
	3	4,98	4,46	3,68	4,01	4,44	2,46	2,97	3,67
FOSB	1	-5,79	-10,39	-10,25	-12,30	-11,10	-10,68	-10,63	-8,83
	2	-4,94	-6,72	-6,87	-9,86	-7,08	-11,82	-9,88	-4,99
	3	-5,33	-8,09	-8,28	-10,05	-8,68	-10,41	-11,59	-6,42
CEACAM6	1	6,42	8,02	8,23	4,50	2,21	10,53	-0,19	-0,17
	2	3,00	4,72	4,29	6,34	3,92	-2,81	0,40	-0,87
	3	6,35	8,04	8,17	4,13	7,83	10,13	5,85	3,62
CES1	1	-5,94	-8,43	-8,13	-8,71	-9,04	-8,39	-7,12	-6,21
	2	-6,12	-8,47	-8,10	-9,34	-7,88	-9,05	-7,40	-7,99
	3	-6,04	-8,68	-7,76	-9,67	-7,71	-7,99	-6,54	-6,59

FIG. 5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B57-RV_T1bG3CIS
B462-RV_CIS
B493-RV_CIS
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B344-RV_T1G3CIS
B152-RV_T2high
B472-RV_T1bG3
B474-RV_TaG3CIS
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B479-0_TaG1
B181-RV_TaG2lowCIS
B121-RV_T1G2low
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B125-RV_TaG1
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B261-RV_TaG1
B72-RV_TaG3
B449-RV_TaG1
B341-RV_T3bG3
B449-0_TaG1
B288-RV2__TaG2high
B288-RV1_TaG2high
B71-RV_TaG2low CIS
B6-RV_TaG2low
CB38-RV
CB30-RV
CB43-RV
CB37-RV
CB25-RV
CB24-RV2
CB24-RV1
CB49-RV
CB45-RV
CB44-RV
B452-0_T2G3CIS
B452-RV_T2G3CIS
B428-RV_T4aG3
B19-RV_T2G3
B109-RV_T2high
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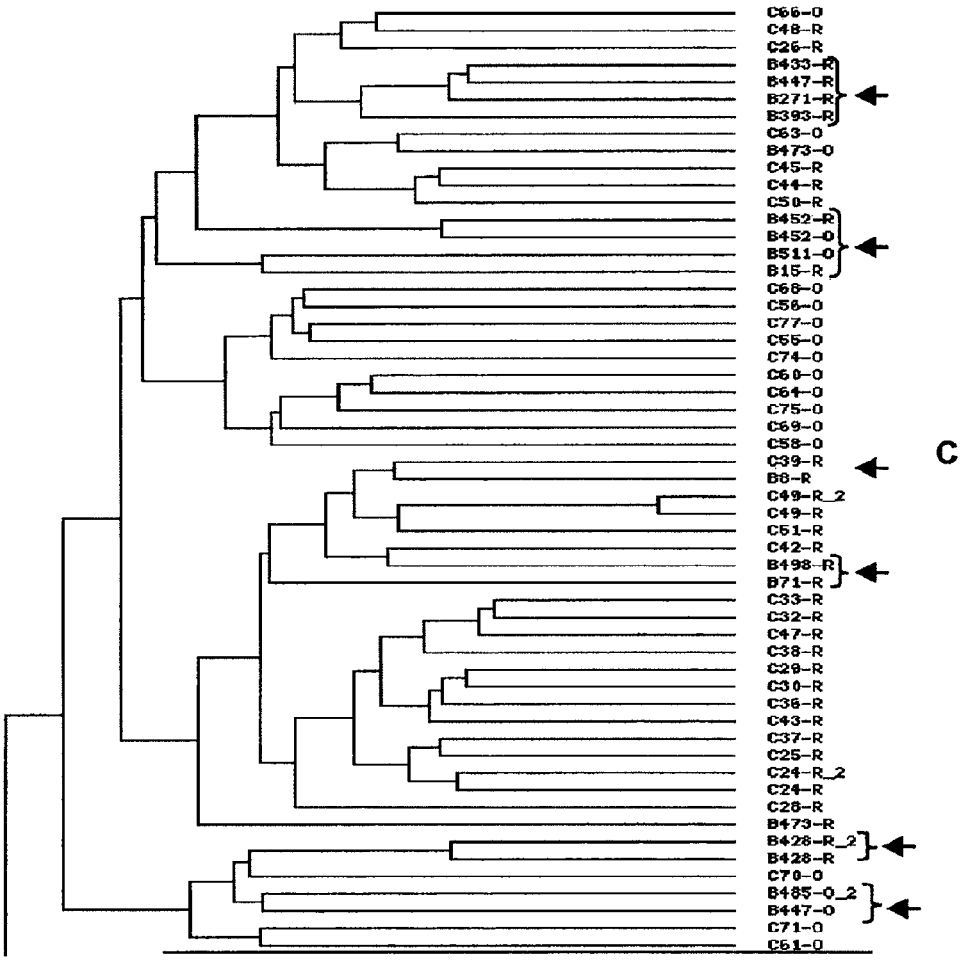
Blood _02-121
Blood _04-94
T_ HG
T_ LG
C
Blood

FIG. 6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C48-R
C26-R
B433-R
B447-R
B271-R
B393-R
C63-O
B473-O
C45-R
C44-R
C50-R
B452-R
B452-O
B511-O
B15-R
C68-O
C56-O
C77-O
C55-O
C74-O
C60-O
C64-O
C75-O
C69-O
C58-O
C39-R
B8-R
C49-R_2
C49-R
C51-R
C42-R
B498-R
B71-R
C33-R
C32-R
C47-R
C38-R
C29-R
C30-R
C36-R
C43-R
C37-R
C25-R
C24-R_2
C24-R
C28-R
B473-R
B428-R_2
B428-R
C70-O
B485-O_2
B447-O
C71-O
C61-O
C

FIG. 7A

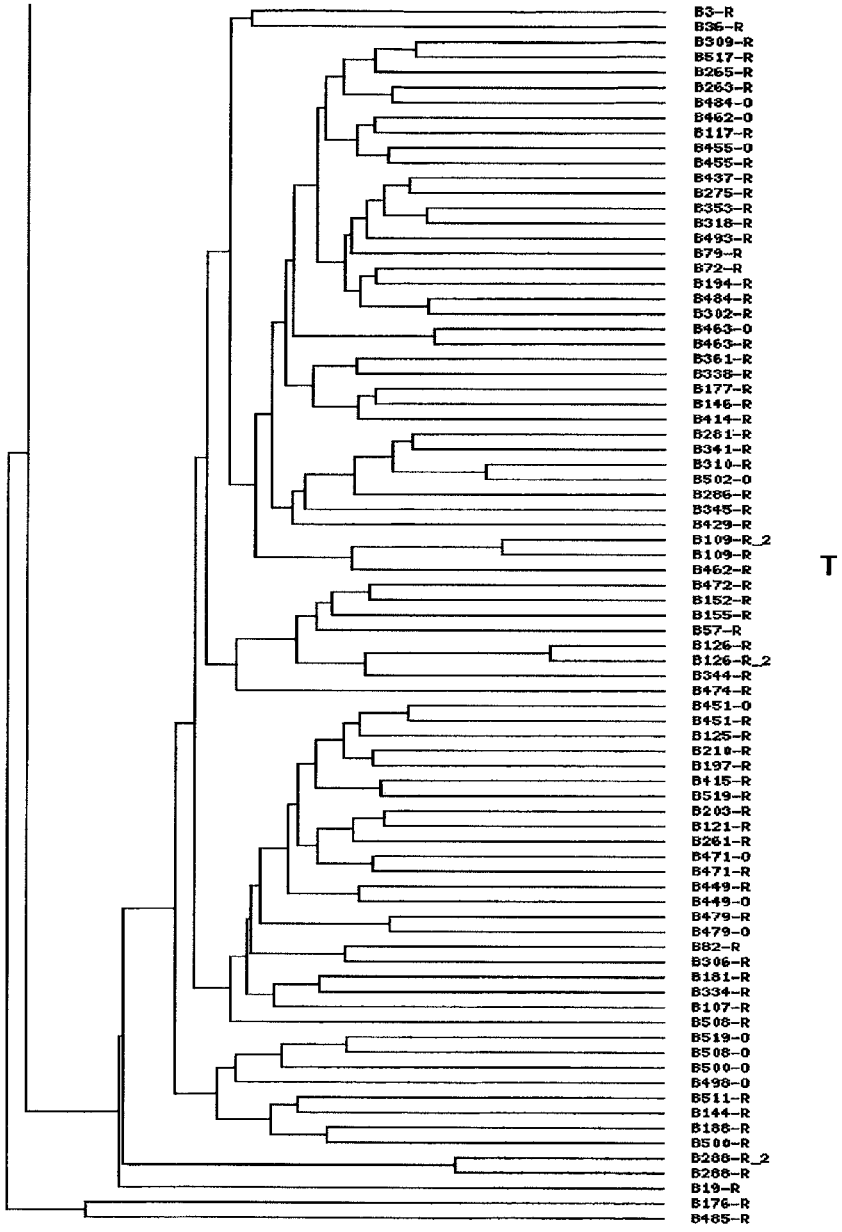
B3-R
B36-R
B309-R
B517-R
B265-R
B263-R
B484-O
B462-O
B117-R
B455-O
B455-R
B437-R
B275-R
B353-R
B318-R
B493-R
B79-R
B72-R
B194-R
B484-R
B302-R
B463-O
B463-R
B361-R
B338-R
B177-R
B146-R
B414-R
B281-R
B341-R
B310-R
B502-O
B286-R
B345-R
B429-R
B109-R_2
B109-R
B462-R
B472-R
B152-R
B155-R
B57-R
B126-R
B126-R_2
B344-R
B474-R
B451-O
B451-R
B125-R
B218-R
B197-R
B415-R
B519-R
B203-R
B121-R
B261-R
B471-O
B471-R
B449-R
B449-O
B479-R
B479-O
B82-R
B306-R
B181-R
B334-R
B107-R
B508-R
B519-O
B508-O
B500-O
B498-O
B511-R
B144-R
B188-R
B500-R
B286-R_2
B286-R
B19-R
B176-R
B485-R
T

FIG. 7B

Gene Symbol	Affymetrix Clone	TaqMan Gene Expression Assay	RefSeq or Gene Bank mRNA	Gene name
ABCA8	204719_at	Hs00200350_m1	NM_007168	ATP-binding cassette sub-family A ABC1 member 8.
ABL1		Hs00245445_m1	2 RefSeqs	V-abl Abelson murine leukemia viral oncogene homolog 1.
ADAM19	209765_at	Hs00224960_m1	2 RefSeqs	A disintegrin and metalloproteinase domain 19 meltrin beta.
ADAMTS1	222162_s_at	Hs00199608_m1	NM_006988	A disintegrin-like and metalloprotease reprolysin type with thrombospondin type 1 motif 1.
AEBP1	201792_at	Hs00371239_m1	NM_001129	AE binding protein 1.
AIM2	206513_at	Hs00175457_m1	NM_004833	Absent in melanoma 2.
AKR1B10	206561_s_at	Hs00252524_m1	NM_020299	Aldo-keto reductase family 1 member B10 aldose reductase.
AL137566	228554_at	Hs00612017_s1	AL137566	
ANK2	202920_at	Hs00153998_m1		Ankyrin 2 neuronal.
ANLN	222608_s_at	Hs00218803_m1	NM_018685	Anillin actin binding protein scraps homolog Drosophila.
ANXA1	233011_at	Hs00167549_m1	NM_000700	Annexin A1.
ANXA10	210143_at	Hs00200464_m1	NM_007193	Annexin A10.
AOC3	204894_s_at	Hs00186647_m1	NM_003734	Amine oxidase copper containing 3 vascular adhesion protein 1.
APOBEC3B	206632_s_at	Hs00358981_m1	NM_004900	Apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3B.
AREG	205239_at	Hs00155832_m1	NM_001657	Amphiregulin schwannoma-derived growth factor.
ARGBP2	225728_at	Hs00221428_m1	NM_021069	Abl-interacting protein ArgBP2.
ASAM	226834_at	Hs00293345_m1	NM_024769	Adipocyte-specific adhesion molecule.
ASPM	219918_s_at	Hs00411505_m1	NM_018136	Asp abnormal spindle-like microcephaly associated Drosophila.

FIG. 8A

ATF3	202672_s_at	Hs00231069_m1		activating transcription factor 3.
ATP5B		Hs00266077_m1	NM_001686	
ATP8B2	226771_at	Hs00393111_m1	NM_020452	ATPase Class I type 8B member 2.
C2orf32	226751_at	Hs00384403_m1	NM_015463	DKFZP566K1924. Cromosome 2 open reading frame 32.
OLFML1	217525_at	Hs00416948_m1	NM_198474	MVAL564 UNQ564. Olfactomedin like-1 OLFML1
BAG2	209406_at	Hs00188716_m1	NM_004282	Apoptosis regulator Bcl-2 protein.
MGC16635	236285_at	Hs00536653_s1	NM_138433	Hypothetical protein BC009980. LOC113730. Kelch repeat.
LOC387882	225105_at	Hs00329098_m1	NM_207376	
BMP5	205431_s_at	Hs00234930_m1	NM_021073	Bone morphogenetic protein 5.
KLF9	203542_s_at	Hs00230918_m1	NM_001206	Basic transcription element binding protein 1.
BUB1B	203755_at	Hs00176169_m1	NM_001211	BUB1 budding uninhibited by benzimidazoles 1 homolog beta yeast.
C10orf3	218542_at	Hs00216688_m1	NM_0181	Chromosome 10 open reading frame 3.
C10orf87	1552566_at	Hs00332436_m1	NM_144587	Chromosome 10 open reading frame 87.
C14orf78	212992_at	Hs00746838_s1		Chromosome 14 open reading frame 78.
C2orf23	204364_s_at	Hs00224761_m1	NM_022912	Chromosome 2 open reading frame 23. TB2/DP1 and HVA22 related protein.
C6orf109	218883_s_at	Hs00209864_m1	NM_015388	KSHV latent nuclear antigen interacting protein 1. KLIP1.
C7	202992_at	Hs00175109_m1	NM_000587	Complement component 7.
CA2	209301_at	Hs00163869_m1	NM_000067	Carbonic anhydrase II.
CALD1	205525_at	Hs00189021_m1		Caldesmon 1.
CAPNS2	223832_s_at	Hs00260517_s1	NM_032330	Calpain small subunit 2.

FIG. 8B

CAV1	212097_at	Hs00184697_m1	NM_001753	Caveolin 1 caveolae protein 22kDa.
CBFA2T1	228827_at	Hs00231702_m1	4 RefSeqs	Core-binding factor runt domain alpha subunit 2; translocated to 1; cyclin D-related.
CCL11	210133_at	Hs00237013_m1	NM_002986	Chemokine C-C motif ligand 11.
CCL18	209924_at	Hs00268113_m1	NM_002988	Chemokine C-C motif ligand 18 pulmonary and activation-regulated.
CCL2	216598_s_at	Hs00234140_m1	NM_002982	Chemokine C-C motif ligand 2.
CCNA2	203418_at	Hs00153138_m1	NM_001237	Cyclin A2.
CCNB1	214710_s_at	Hs00259126_m1	NM_031966	Cyclin B1.
CCNB2	202705_at	Hs00270424_m1	NM_004701	cyclin B2.
CCND1		Hs00277039_m1	NM_053056	cyclin D1 PRAD1: parathyroid adenomatosis 1.
CCND2	200953_s_at	Hs00277041_m1	NM_001759	Cyclin D2. Regulation of cell cycle. Cytokinesis.
CCNE1		Hs00233356_m1	2 RefSeqs	Cyclin E1.
CDC2	210559_s_at	Hs00364293_m1	NM_001786	Cell division cycle 2 G1 to S and G2 to M.
CDC20	202870_s_at	Hs00415851_g1	NM_001255	CDC20 cell division cycle 20 homolog S. cerevisiae.
CDC6	203967_at	Hs00154374_m1	NM_001254	CDC6 cell division cycle 6 homolog S. cerevisiae.
CDCA1	223381_at	Hs00230097_m1	2 RefSeqs	Cell division cycle associated 1.
CDCA3	223307_at	Hs00229905_m1	NM_031299	Cell division cycle associated 3.
CDH1		Hs00170423_m1	NM_004360	Cadherin 1 type 1 E-cadherin epithelial.
CDH11	207173_x_at	Hs00156438_m1	2 RefSeqs	Cadherin 11 type 2 OB-cadherin osteoblast.
CDH19	206898_at	Hs00253534_m1	NM_021153	Cadherin 19 type 2.
CDKN2A		Hs00233365_m1	3 RefSeqs	Cyclin-dependent kinase inhibitor 2A melanoma p16 inhibits CDK4.
CDKN2B	236313_at	Hs00365249_m1	NM_078487	Cyclin-dependent kinase inhibitor 2B p15 inhibits CDK4.

FIG. 8C

CDKN3	209714_s_at	Hs00193192_m1	NM_005192	Cyclin-dependent kinase inhibitor 3 CDK2-associated dual specificity phosphatase.
CEACAM6	211657_at	Hs00366002_m1	NM_002483	Carcinoembryonic antigen-related cell adhesion molecule 6 non-specific cross reacting antigen.
CEACAM7	206199_at	Hs00185152_m1	NM_006890	Carcinoembryonic antigen-related cell adhesion molecule 7.
CENPA	204962_s_at	Hs00156455_m1	NM_001809	Centromere protein A 17kDa.
CENPF	207828_s_at	Hs00193201_m1	NM_016343	Centromere protein F 350/400ka mitosin.
CFL2	224663_s_at	Hs00368395_g1		cofilin 2 muscle.
ChGn	219049_at	Hs00218054_m1	NM_018371	Chondroitin beta1 4 N-acetylgalactosaminyltransferase.
CHI3L1	209395_at	Hs00609691_m1	NM_001276	Chitinase 3-like 1 cartilage glycoprotein-39.
CKS2	204170_s_at	Hs00854958_g1	NM_001827	CDC28 protein kinase regulatory subunit 2.
CLCA2	206165_s_at	Hs00197957_m1	NM_006536	Chloride channel calcium activated family member 2.
CLCN3	201733_at	Hs00156527_m1	NM_001829	Chloride channel 3.
CLIC3	219529_at	Hs00362166_g1	NM_004669	Chloride intracellular channel 3.
CLIC4	201560_at	Hs00749895_s1	NM_013943	Chloride intracellular channel 4.
COL14A1	212865_s_at	Hs00385388_m1		Collagen type XIV alpha 1 undulin.
COL15A1	203477_at	Hs00266332_m1	NM_001855	Collagen type XV alpha 1.
COL1A2	202403_s_at	Hs00164099_m1	NM_000089	Collagen type I alpha 2.
COL3A1	215076_s_at	Hs00164103_m1	NM_000090	Collagen type III alpha 1 Ehlers-Danlos syndrome type IV autosomal dominant.
COL5A2	221730_at	Hs00169768_m1	NM_000393	Collagen type V alpha 2. Cell growth and/or maintenance.
COL6A1	213428_s_at	Hs00242448_m1	NM_001848	Collagen type VI alpha 1. Cell adhesion.
COL6A2	209156_s_at	Hs00242484_m1	NM_001849	Collagen type VI alpha 2. Cell-cell adhesion.

FIG. 8D

COLEC12	221019_s_at	Hs00560477_m1	2 RefSeqs	Collectin sub-family member 12.
CPA3	205624_at	Hs00157019_m1	NM_001870	Carboxypeptidase A3 mast cell.
CPE	201116_s_at	Hs00175676_m1	NM_001873	Carboxypeptidase E.
CRH	205630_at	Hs00174941_m1	NM_000756	Corticotropin releasing hormone.
CRTAC1	221204_s_at	Hs00216208_m1	NM_018058	Cartilage acidic protein 1.
CTGF	209101_at	Hs00170014_m1	NM_001901	Connective tissue growth factor.
CTSE	205927_s_at	Hs00157213_m1		cathepsin E.
CTTN		Hs00193322_m1	2 RefSeqs	Cortactin.
VSIG2	228232_s_at	Hs00204823_m1	NM_014312	Cortical thymocyte receptor X. laevis CTX like.
CUGBP2	202157_s_at	Hs00272516_m1	NM_006561	CUG triplet repeat RNA binding protein 2.
CXCL12	209687_at	Hs00171022_m1		Chemokine C-X-C motif ligand 12 stromal cell-derived factor 1.
CXCR4	217028_at	Hs00237052_m1	NM_003467	Chemokine C-X-C motif receptor 4.
CYBRD1	222453_at	Hs00227411_m1	NM_024843	Cytochrome b reductase 1. Electron transport.
CYP24A1		Hs00167999_m1	NM_000782	Cytochrome P450 family 24 subfamily A polypeptide 1.
CYR61	201289_at	Hs00155479_m1	NM_001554	Cysteine-rich angiogenic inducer 61. Regulation of cell growth.
D4S234E	209569_x_at	Hs00205189_m1	NM_014392	DNA segment on chromosome 4 unique 234 expressed sequence.
DBC1		Hs00180893_m1	NM_014618	Deleted in bladder cancer 1.
DCN	209335_at	Hs00266491_m1		Decorin. Organogenesis.
DF	205382_s_at	Hs00157263_m1	NM_001928	D component of complement adipsin.
DKFZp434B044	221541_at	Hs00230322_m1	NM_031476	hypothetical protein DKFZp434B044.

FIG. 8E

DKFZp564O0823	225809_at	Hs00209875_m1	NM_015393	DKFZP564O0823 protein
DKFZp586H2123	213661_at	Hs00405837_m1	NM_015430	DKFZP586H2123 protein.
DKK1	204602_at	Hs00183740_m1	NM_012242	Dickkopf homolog 1 Xenopus laevis.
DLG7	203764_at	Hs00207323_m1	NM_014750	Discs large homolog 7 Drosophila.
DOC1	1554966_a_at	Hs00706279_s1		Downregulated in ovarian cancer 1.
DPT	213068_at	Hs00170030_m1	NM_001937	dermatopontin. Cell adhesion.
DPYSL3	201431_s_at	Hs00181665_m1	NM_001387	Dihydropyrimidinase-like 3.
DSCR1L1	203498_at	Hs00195165_m1	NM_005822	Down syndrome critical region gene 1-like 1.
DTR	203821_at	Hs00181813_m1	NM_001945	Diphtheria toxin receptor heparin-binding epidermal growth factor-like growth factor.
DUSP1	201041_s_at	Hs00610256_g1	NM_004417	Dual specificity phosphatase 1.
E2F3		Hs00605457_m1	NM_001949	E2F transcription factor 3.
EBF	227646_at	Hs00395513_m1	NM_024007	Early B-cell factor.
ECRG4	223623_at	Hs00260897_m1	NM_032411	Esophageal cancer related gene 4 protein.
ECT2	219787_s_at	Hs00216455_m1	NM_018098	Epithelial cell transforming sequence 2 oncogene.
EDNRA	204464_s_at	Hs00609865_m1	NM_001957	Endothelin receptor type A.
EGR1	201694_s_at	Hs00152928_m1	NM_001964	early growth response 1.
ENTPD3	206191_at	Hs00154325_m1	NM_001248	Ectonucleoside triphosphate diphosphohydrolase 3.
EPHA3	206070_s_at	Hs00178327_m1	NM_005233	EphA3.
EPHA7	229288_at	Hs00177891_m1	NM_004440	EphA7.
ERBB2		Hs00170433_m1	2 RefSeqs	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2 neuro/glioblastoma derived oncogene homolog avian.

FIG. 8F

F3	204363_at	Hs00175225_m1	NM_001993	Coagulation factor III thromboplastin tissue factor.
FABP4	203980_at	Hs00609791_m1	NM_001442	Fatty acid binding protein 4 adipocyte.
FABP6	210445_at	Hs00155029_m1	NM_001445	Fatty acid binding protein 6 ileal gastrotropin.
FAP	209955_s_at	Hs00189476_m1	NM_004460	Fibroblast activation protein alpha.
FBLN1	202994_s_at	Hs00242545_m1	4 RefSeqs	Fibulin 1.
FBLN5	203088_at	Hs00197064_m1	NM_006329	Fibulin 5.
FBN1	202766_s_at	Hs00171191_m1	NM_000138	Fibrillin 1 Marfan syndrome.
FBN2	203184_at	Hs00417208_m1	NM_001999	Fibrillin 2 congenital contractural arachnodactyly.
FBXO32	225328_at	Hs00369714_m1		F-box only protein 32.
FEN1	204768_s_at	Hs00748727_s1	NM_004111	Flap structure-specific endonuclease 1.
FGF3		Hs00173742_m1	NM_005247	Fibroblast growth factor 3 murine mammary tumor virus integration site v-int-2 oncogene homolog.
FGFR1		Hs00241111_m1	9 RefSeqs	Fibroblast growth factor receptor 1 fms-related tyrosine kinase 2 Pfeiffer syndrome.
FGFR3	204380_s_at	Hs00179829_m1	2 RefSeqs	Fibroblast growth factor receptor 3 achondroplasia thanatophoric dwarfism.
FGL2	227265_at	Hs00173847_m1	NM_006682	
FLJ10159	218974_at	Hs00215979_m1	NM_018013	Hypothetical protein FLJ10159
FLJ10719	213007_at	Hs00289551_m1	NM_018193	Hipothetycal protein FLJ10719
FLJ11029	228273_at	Hs00383634_m1	NM_018304	Hypothetical protein FLJ11029
FLJ13710	222835_at	Hs00388227_m1	NM_024817	Hypothetical protein FLJ13710.
TSC	218872_at	Hs00215487_m1	NM_017899	Hipothetycal protein FLJ20607
FLJ20701	219093_at	Hs00374054_g1		Hypothetical protein FLJ20701

FIG. 8G

FLJ21986	228728_at	Hs00227735_m1	NM_024913	Hypothetical protein FLJ21986
FLJ23191	219747_at	Hs00375503_m1	NM_024574	Hypothetical protein FLJ23191
SPOCD1	235417_at	Hs00375905_m1	NM_144569	Hipothetycal protein FLJ2234.Transcription elongation factor S-II central region.
FLJ31052	238452_at	Hs00708284_s1	NM_152378	Hypothetical protein FLJ31052
FLJ32569	239929_at	Hs00611179_m1	NM_152491	Proteolysis and peptidolysis.
FLJ38736	227174_at	Hs00419054_m1	NM_182758	Hypothetical protein FLJ38736.
FN1	211719_x_at	Hs00365058_m1	7 RefSeqs	Fibronectin 1.
FNBP1	212288_at	Hs00390705_m1	NM_015033	Formin binding protein 1.
FOS	209189_at	Hs00170630_m1	NM_005252	V-fos FBJ murine osteosarcoma viral oncogene homolog.
FOSB	202768_at	Hs00171851_m1	NM_006732	FBJ murine osteosarcoma viral oncogene homolog B.
FOXF1	205935_at	Hs00230962_m1	NM_001451	Forkhead box F1.
FOXM1	202580_x_at	Hs00153543_m1	2 RefSeqs	Forkhead box M1.
FYN	210105_s_at	Hs00176628_m1	3 RefSeqs	FYN oncogene related to SRC FGR YES.
FZD7	203706_s_at	Hs00275833_s1	NM_003507	Frizzled homolog 7 Drosophila.
GATA6	210002_at	Hs00232018_m1	NM_005257	GATA binding protein 6.
GEM	204472_at	Hs00170633_m1	NM_005261	GTP binding protein overexpressed in skeletal muscle.
GGH	203560_at	Hs00608257_m1	NM_003878	Gamma-glutamyl hydrolase conjugase folylpolygammaglutamyl hydrolase.
GHR	205498_at	Hs00174872_m1	NM_000163	Growth hormone receptor.
GJB2	223278_at	Hs00269615_s1	NM_004004	Gap junction protein beta 2 26kDa connexin 26. Cell-cell signaling.
GJB6	231771_at	Hs00272726_s1	NM_006783	Gap junction protein beta 6 connexin 30.

FIG. 8H

GKN1	220191_at	Hs00219734_m1	NM_019617	Gastrokine 1.
GMNN	218350_s_at	Hs00210707_m1	NM_015895	Geminin DNA replication inhibitor.
GPC6	227059_at	Hs00170677_m1	NM_005708	Glypican 6. Cell growth and/or maintenance.
GPM6B	209167_at	Hs00383529_m1	4 RefSeqs	Glycoprotein M6B.
GPR124	221814_at	Hs00262150_m1	NM_032777	G protein-coupled receptor 124.
GREM1	218469_at	Hs00171951_m1	NM_013372	Gremlin 1 homolog cysteine knot superfamily Xenopus laevis.
GSN		Hs00609276_m1	2 RefSeqs	Gelsolin amyloidosis Finnish type.
GULP1	204235_s_at	Hs00169604_m1	NM_016315	GULP engulfment adaptor PTB domain containing 1.
GUSB		Hs99999908_m1	NM_000181	
H19	224646_x_at	Hs00399294_g1		H19 imprinted maternally expressed untranslated mRNA.
HEPH	203903_s_at	Hs00207710_m1		Hephaestin.
CFH	213800_at	Hs00164830_m1	NM_000186	H factor 1 complement.
HIST1H1C	209398_at	Hs00271185_s1	NM_005319	Histone 1 H1c.
HIST1H2BD	209911_x_at	Hs00371070_m1	NM_138720	Histone 1 H2bd.
HIST1H2BG	210387_at	Hs00374317_s1	NM_003518	Histone 1 H2bg.
HIST2H2AA	214290_s_at	Hs00358508_s1	NM_003516	Histone 2 H2aa.
HIST2H2BE	202708_s_at	Hs00269023_s1	NM_003528	Histone 2 H2be.
HMGB2	208808_s_at	Hs00357789_g1	NM_002129	High-mobility group box 2.
HMOX1	203665_at	Hs00157965_m1	NM_002133	Heme oxygenase decycling 1.
HN1	217755_at	Hs00602957_m1	3 RefSeqs	Hematological and neurological expressed 1.

FIG. 8I

HOP	211597_s_at	Hs00261238_m1	3 RefSeqs	Homeodomain-only protein.
HOXB6	205366_s_at	Hs00255831_s1	3 RefSeqs	Homeo box B6.
HOXD4	205522_at	Hs00429605_m1	NM_014621	Homeo box D4.
HPRT		Hs99999909_m1	NM_000194	
HRAS		Hs00610483_m1	NM_005343	
HSPC150	223229_at	Hs00204359_m1	NM_014176	HSPC150 protein similar to ubiquitin-conjugating enzyme.
HSPCB		Hs00607336_gH	NM_007355	
HTR1F		Hs00265296_s1	NM_000866	5-hydroxytryptamine serotonin receptor 1F.
IER3	201631_s_at	Hs00174674_m1	NM_003897	Immediate early response 3.
IGF1	209541_at	Hs00153126_m1	NM_000618	Insulin-like growth factor 1 somatomedin C.
IGF2	210881_s_at	Hs00171254_m1	NM_000612	Insulin-like growth factor 2 somatomedin A.
IGFBP3	210095_s_at	Hs00181211_m1	NM_000598	Insulin-like growth factor binding protein 3.
IGFBP5	211959_at	Hs00181213_m1	NM_000599	Insulin-like growth factor binding protein 5.
IGHM	216491_x_at	Hs03378512_m1		Immunoglobulin heavy constant mu.
IL6	205207_at	Hs00174131_m1	NM_000600	Interleukin 6 interferon beta 2.
INA	204465_s_at	Hs00190771_m1	NM_032727	Internexin neuronal intermediate filament protein alpha.
INHBA	210511_s_at	Hs00170103_m1	NM_002192	Inhibin beta A activin A activin AB alpha polypeptide.
IQGAP3	229490_s_at	Hs00603642_m1	NM_178229	IQ motif containing GTPase activating protein 3. Ras GTPase-activating protein.
ITGA8	AA903473	Hs00233321_m1	NM_003638	
ITM2A	202746_at	Hs00191609_m1	NM_004867	Integral membrane protein 2A.

FIG. 8J

JAM2	229127_at	Hs00221894_m1	NM_021219	Junctional adhesion molecule 2.
JAM3	212813_at	Hs00230289_m1	NM_032801	Junctional adhesion molecule 3.
JUNB		Hs00357891_s1	NM_002229	Jun B proto-oncogene.
KCNG1	214595_at	Hs00158410_m1	NM_002237	Potassium voltage-gated channel subfamily G member 1.
KDELR3	204017_at	Hs00423556_m1	2 RefSeqs	KDEL Lys-Asp-Glu-Leu endoplasmic reticulum protein retention receptor 3.
KIAA0101	202503_s_at	Hs00207134_m1	NM_014736	KIAA0101 gene product
KIAA0186	206102_at	Hs00221421_m1	NM_021067	KIAA0186 gene product.
KIAA0992	200897_s_at	Hs00363101_m1	NM_016081	Palladin.
KIF11	204444_at	Hs00189698_m1	NM_004523	Kinesin family member 11.
KIF14	206364_at	Hs00208408_m1	NM_014875	Kinesin family member 14.
KIF20A	218755_at	Hs00194882_m1	NM_005733	Kinesin family member 20A.
KIF2C	209408_at	Hs00199232_m1	NM_006845	Kinesin family member 2C.
KIF4A	218355_at	Hs00602211_g1	NM_012310	Kinesin family member 4A.
KISS1		Hs00158486_m1	NM_002256	KiSS-1 metastasis-suppressor.
KLHL7	220239_at	Hs00375239_m1	NM_018846	Kelch-like 7 Drosophila.
KPNA2	201088_at	Hs00818252_g1	NM_002266	Karyopherin alpha 2 RAG cohort 1 importin alpha 1.
KRT14	209351_at	Hs00265033_m1	NM_000526	Keratin 14 epidermolysis bullosa simplex Dowling-Meara Koebner.
KRT20	213953_at	Hs00300643_m1	NM_019010	Keratin 20.
KRT7	209016_s_at	Hs00818825_m1	NM_005556	Keratin 7.
LAF4	227198_at	Hs00171448_m1	NM_002285	Lymphoid nuclear protein related to AF4.

FIG. 8K

LEPR	209894_at	Hs00174497_m1	NM_002303	Leptin receptor.
LGALS1	201105_at	Hs00169327_m1	NM_002305	Lectin galactoside-binding soluble 1 galectin 1.
LIFR	225575_at	Hs00158730_m1	NM_002310	Leukemia inhibitory factor receptor.
LOC83468	227070_at	Hs00229917_m1	NM_031302	Gycosyltransferase.
LOX	215446_s_at	Hs00184700_m1	NM_002317	Lysyl oxidase
LPPR4	213496_at	Hs00322721_m1	NM_014839	Plasticity related gene 1.
LUM	229554_at	Hs00158940_m1	NM_002345	Lumican.
LY6D	206276_at	Hs00170353_m1	NM_003695	Lymphocyte antigen 6 complex locus D.
MAD2L1	203362_s_at	Hs00829154_g1	NM_002358	MAD2 mitotic arrest deficient-like 1 yeast.
MAGEA3	209942_x_at	Hs00366532_m1	NM_005362	Melanoma antigen family A 3.
MAGEA9	210437_at	Hs00245619_s1	NM_005365	Melanoma antigen family A 9.
MAMDC2	228885_at	Hs00299196_m1	NM_153267	MAM domain containing 2.
MAN1C1	214180_at	Hs00220595_m1	NM_020379	Mannosidase alpha class 1C member 1.
MAP1B	226084_at	Hs00195487_m1	2 RefSeqs	Microtubule-associated protein 1B.
MCM10	220651_s_at	Hs00218560_m1	2 RefSeqs	Minichromosome maintenance deficient 10 S.
MCM2	202107_s_at	Hs00170472_m1	NM_004526	MCM2 minichromosome maintenance deficient 2 mitotin S.
MDM2		Hs00242813_m1	3 RefSeqs	Mdm2 transformed 3T3 cell double minute 2 p53 binding protein mouse.
MELK	204825_at	Hs00207681_m1	NM_014791	Maternal embryonic leucine zipper kinase.
MFAP2	203417_at	Hs00250064_m1	2 RefSeqs	Microfibrillar-associated protein 2.
MFAP4	212713_at	Hs00412974_m1	NM_002404	Microfibrillar-associated protein 4.

FIG. 8L

TUBB6	209191_at	Hs00603164_m1	NM_032525	Tubulin beta MGC4083.
MGC45780	229839_at	Hs00382351_m1	NM_173833	Hypothetical protein.
MGC57827	225834_at	Hs00822131_m1	NM_207418	Similar to RIKEN cDNA 2700049P18 gene.
MGP	202291_s_at	Hs00179899_m1	NM_000900	Matrix Gla protein.
MKI67	212021_s_at	Hs00606991_m1	NM_002417	Antigen identified by monoclonal antibody Ki-67.
MMD	203414_at	Hs00202450_m1	NM_012329	Monocyte to macrophage differentiation-associated.
MMP1	204475_at	Hs00233958_m1	NM_002421	Matrix metalloproteinase 1 interstitial collagenase.
MMP12	204580_at	Hs00159178_m1	NM_002426	Matrix metalloproteinase 12 macrophage elastase.
MRS2L	218538_s_at	Hs00252895_m1	NM_020662	MRS2-like magnesium homeostasis factor S. cerevisiae.
MRVI1	226047_at	Hs00180652_m1		Murine retrovirus integration site 1 homolog.
MSRB3	225782_at	Hs00827017_m1	NM_198080	Methionine sulfoxide reductase B3
MST1R	205455_at	Hs00234013_m1	NM_002447	Macrophage stimulating 1 receptor c-met-related tyrosine kinase.
MTHFD2	201761_at	Hs00759197_s1	NM_006636	Methylene tetrahydrofolate dehydrogenase NAD+ dependent methenyltetrahydrofolate cyclohydrolase.
MUC7		Hs00379529_m1	NM_152291	Mucin 7 salivary.
MYADM	225673_at	Hs00414763_m1	NM_138373	Myeloid-associated differentiation marker
MYBPC1	214087_s_at	Hs00159451_m1		Myosin binding protein C slow type.
MYC		Hs00153408_m1	NM_002467	V-myc myelocytomatosis viral oncogene homolog avian.
NEK2	204641_at	Hs00601227_mH	NM_002497	NIMA never in mitosis gene a-related kinase 2.
NEXN	1552309_a_at	Hs00332124_m1	NM_144573	Nexilin F actin binding protein.
NFIA	226806_s_at	Hs00325656_m1	NM_005595	

FIG. 8M

NFIB	213029_at	Hs00232149_m1	NM_005596	Nuclear factor I/B.
NFIL3	203574_at	Hs00356605_g1	NM_005384	Nuclear factor interleukin 3 regulated.
FLJ22595	220468_at	Hs00540047_s1	NM_025047	Hipothetycal protein FLJ22595
NMES1	223484_at	Hs00260902_m1		Normal mucosa of esophagus specific 1. Nucleus.
NNMT	202237_at	Hs00196287_m1	NM_006169	Nicotinamide N-methyltransferase.
NOPE	227870_at	Hs00326335_m1	NM_020962	Likely ortholog of mouse neighbor of Punc E11.
NQO1	201468_s_at	Hs00168547_m1	NM_000903	NADPH dehydrogenase quinone 1.
NR2F1	209505_at	Hs00818842_m1	NM_005654	Nuclear receptor subfamily 2 group F member 1.
NR4A1	202340_x_at	Hs00172437_m1	NM_173157	Nuclear receptor subfamily 4 group A member 1.
NR4A2	204621_s_at	Hs00428691_m1	4 RefSeqs	Nuclear receptor subfamily 4 group A member 2.
NR4A3	209959_at	Hs00175077_m1		Nuclear receptor subfamily 4 group A member 3.
NTNG2		Hs00287286_m1	NM_032536	Netrin G2.
NUSAP1	218039_at	Hs00153533_m1	NM_016359	Nucleolar and spindle associated protein 1.
OAS1	202869_at	Hs00242943_m1	2 RefSeqs	2' 5'-oligoadenylate synthetase 1 40/46kDa.
OLFML3	218162_at	Hs00220180_m1	NM_020190	Olfactomedin-like 3.
OSR2	213568_at	Hs00369588_m1	NM_053001	Odd-skipped-related 2A protein.
		Hs00211916_m1	NM_016205	
PDGFC				
PDGFRA	203131_at	Hs00183486_m1	NM_006206	Platelet-derived growth factor receptor alpha polypeptide.
PDLIM3	209621_s_at	Hs00205533_m1	NM_014476	PDZ and LIM domain 3.
PDZRN3	212915_at	Hs00392900_m1		PDZ domain containing RING finger 3.

FIG. 8N

PEG10	212094_at	Hs00248288_s1	NM_015068	Paternally expressed 10.
PFKFB3	202464_s_at	Hs00190079_m1	NM_004566	6-phosphofructo-2-kinase/fructose-2 6-biphosphatase 3.
Pfs2	221521_s_at	Hs00211479_m1	NM_016095	DNA replication complex GINS protein PSF2. DNA replication.
PGM5	226303_at	Hs00222671_m1	NM_021965	phosphoglucomutase 5. Cell-cell adherens junction.
PLA2G2A	203649_s_at	Hs00179898_m1	NM_000300	Phospholipase A2 group IIA platelets synovial fluid. Lipid catabolism.
PLAGL1	209318_x_at	Hs00243030_m1	NM_002656	Pleiomorphic adenoma gene-like 1.
PLCB4	203895_at	Hs00168656_m1		Phospholipase C beta 4.
PLEKHC1	209209_s_at	Hs00235033_m1	NM_006832	Pleckstrin homology domain containing family C with FERM domain member 1.
PLN	204939_s_at	Hs00160179_m1	NM_002667	phospholamban.
PLSCR4	218901_at	Hs00220482_m1	NM_020353	Phospholipid scramblase 4.
PMP22	210139_s_at	Hs00165556_m1		Peripheral myelin protein 22.
POLQ	219510_at	Hs00198196_m1	2 RefSeqs	Polymerase DNA directed theta.
POSTN	1555778_a_at	Hs00170815_m1	NM_006475	Periostin osteoblast specific factor.
POU1F1		Hs00230821_m1	NM_000306	POU domain class 1 transcription factor 1 Pit1 growth hormone factor 1.
PPIA o CYC		Hs99999904_m1	3 RefSeqs	
PPP1R12B	201957_at	Hs00364078_m1		Protein phosphatase 1 regulatory inhibitor subunit 12B.
PPP1R14D	220082_at	Hs00214613_m1	NM_017726	Protein phosphatase 1 regulatory inhibitor subunit 14D.
PRC1	218009_s_at	Hs00187740_m1		Protein regulator of cytokinesis 1.
PRKAR2B	203680_at	Hs00176966_m1	NM_002736	Protein kinase cAMP-dependent regulatory type II beta.
PRL		Hs00168730_m1	NM_000948	Prolactin.

FIG. 8O

PSAT1	223062_s_at	Hs00795278_mH	2 RefSeqs	Phosphoserine aminotransferase 1.
PTCH		Hs00181117_m1	NM_000264	Patched homolog Drosophila.
PTEN		Hs00829813_s1	NM_000314	Phosphatase and tensin homolog mutated in multiple advanced cancers 1.
PTGS2	1554997_a_at	Hs00153133_m1	NM_000963	Prostaglandin-endoperoxide synthase 2 prostaglandin G/H synthase and cyclooxygenase.
PTN	211737_x_at	Hs00383235_m1	NM_002825	Pleiotrophin heparin binding growth factor 8 neurite growth-promoting factor 1.
PTPRC		Hs00236304_m1	3 RefSeqs	
PTRF	1557938_s_at	Hs00396859_m1	NM_012232	Polymerase I and transcript release factor abans AL545542.
RAB23	229504_at	Hs00212407_m1		RAB23 member RAS oncogene family.
RACGAP1	222077_s_at	Hs00374747_m1	NM_013277	Rac GTPase activating protein 1.
RAI2	219440_at	Hs00253960_s1	NM_021785	Retinoic acid induced 2.
RAMP	218585_s_at	Hs00212788_m1	NM_016448	RA-regulated nuclear matrix-associated protein.
RASL12	219167_at	Hs00275429_m1	NM_016563	RAS-like family 12.
RB1		Hs00153108_m1	NM_000321	Retinoblastoma 1 including osteosarcoma.
RBM24	235004_at	Hs00290607_m1	NM_153020	RNA binding motif protein 24.
RECK	205407_at	Hs00221638_m1	NM_021111	Reversion-inducing-cysteine-rich protein with kazal motifs.
RFC3	204127_at	Hs00161357_m1	2 RefSeqs	Replication factor C activator 1 3 38kDa. DNA replication.
RGS1	216834_at	Hs00175260_m1	NM_002922	Regulator of G-protein signalling 1. Immune response.
RNASE4	213397_x_at	Hs00377763_m1		Ribonuclease RNase A family 4.
RODH	205700_at	Hs00366258_m1	NM_003725	3-hydroxysteroid epimerase.

FIG. 8P

RPESP	235210_s_at	Hs00541931_m1	NM_153225	RPE-spondin.
RRM2	209773_s_at	Hs00357247_g1	NM_001034	Ribonucleotide reductase M2 polypeptide.
S100A10	238909_at	Hs00741221_m1	NM_002966	S100 calcium binding protein A10 annexin II ligand calpactin I light polypeptide p11.
SBLF	213413_at	Hs00538997_m1		Stoned B-like factor.
SCN7A	228504_at	Hs00161546_m1	NM_002976	Sodium channel voltage-gated type VII alpha.
SELL	204563_at	Hs00174151_m1	NM_000655	Selectin L lymphocyte adhesion molecule 1.
SELM	226051_at	Hs00369741_m1	NM_080430	Selenoprotein M.
SERPINB3	209719_x_at	Hs00199468_m1	NM_006919	Serine or cysteine proteinase inhibitor clade B ovalbumin member 3.
SETBP1	205933_at	Hs00210209_m1	NM_015559	SET binding protein 1.
SFRP1	202037_s_at	Hs00610060_m1	NM_003012	Secreted frizzled-related protein 1.
SLC1A6	1554593_s_at	Hs00192604_m1	NM_005071	Solute carrier family 1 high affinity aspartate/glutamate transporter member 6.
SLIT2	209897_s_at	Hs00191193_m1	NM_004787	slit homolog 2 (Drosophila).
SMAD6		Hs00178579_m1	NM_005585	SMAD mothers against DPP homolog 6 Drosophila.
SMOC2	223235_s_at	Hs00405777_m1	NM_022138	SPARC related modular calcium binding 2.
SNX10	218404_at	Hs00203362_m1	NM_013322	Sorting nexin 10.
SOCS3	227697_at	Hs00269575_s1	NM_003955	Suppressor of cytokine signaling 3.
SOX4	213668_s_at	Hs00268388_s1	NM_003107	SRY sex determining region Y-box 4.
SOX9	202935_s_at	Hs00165814_m1	NM_000346	SRY sex determining region Y-box 9 campomelic dysplasia autosomal sex-reversal
SPARCL1	200795_at	Hs00190740_m1	NM_004684	SPARC-like 1 mast9 hevin.
SPON1	209436_at	Hs00323883_m1		Spondin 1 extracellular matrix protein.

FIG. 8Q

SPP1	209875_s_at	Hs00167093_m1	NM_000582	Secreted phosphoprotein 1 osteopontin bone sialoprotein I early T-lymphocyte activation 1.
SPRR3	218990_s_at	Hs00271304_m1	NM_005416	Small proline-rich protein 3. Structural molecule activity.
SRPX	204955_at	Hs00196867_m1	NM_006307	Sushi-repeat-containing protein X-linked.
STC1	230746_s_at	Hs00174970_m1	NM_003155	Stanniocalcin 1.
STK6	208079_s_at	Hs00269212_m1	NM_003600	Serine/threonine kinase 6.
STN2	235852_at	Hs00263833_m1	NM_033104	Stonin 2.
SULF1	212354_at	Hs00290918_m1	NM_015170	Sulfatase 1.
SULT1E1	219934_s_at	Hs00193690_m1	NM_005420	Sulfotransferase family 1E estrogen-preferring member 1.
TCF21	204931_at	Hs00162646_m1		Transcription factor 21.
TCF8	212764_at	Hs00611018_m1	NM_030751	Transcription factor 8 represses interleukin 2 expression.
TCN1	205513_at	Hs00169055_m1	NM_001062	transcobalamin I (vitamin B12 binding protein, R binder family).
TEAD2	226408_at	Hs00366217_m1	NM_003598	TEA domain family member 2.
TEGT		Hs00162661_m1	NM_003217	
TERT		Hs00162669_m1	2 RefSeqs	Telomerase reverse transcriptase.
TGFB1I1	209651_at	Hs00210887_m1	NM_015927	Transforming growth factor beta 1 induced transcript 1.
TIMP2	224560_at	Hs00234278_m1	NM_003255	Tissue inhibitor of metalloproteinase 2.
TK1	1554408_a_at	Hs00177406_m1	NM_003258	Thymidine kinase 1 soluble.
TMPO	203432_at	Hs00162842_m1	NM_003276	Thymopoietin. DNA binding.
TNA	205200_at	Hs00162844_m1	NM_003278	Tetranectin.
TNC	201645_at	Hs00233648_m1	NM_002160	Tenascin C.

FIG. 8R

TNRC9	216623_x_at	Hs00300355_m1		Trinucleotide repeat containing 9.
TOP2A	201291_s_at	Hs00172214_m1	NM_001067	Topoisomerase DNA II alpha 170kDa.
TOPK	219148_at	Hs00218544_m1	NM_018492	T-LAK cell-originated protein kinase.
TP53		Hs00153349_m1	NM_000546	Tumor protein p53 Li-Fraumeni syndrome.
TPX2	210052_s_at	Hs00201616_m1	NM_012112	TPX2 microtubule-associated protein homolog Xenopus laevis.
TRIP13	204033_at	Hs00188500_m1	NM_004237	Thyroid hormone receptor interactor 13.
TSPAN-2	227236_at	Hs00194836_m1	NM_005725	Tetraspan 2. Cell motility.
TTK	204822_at	Hs00177412_m1	NM_003318	TTK protein kinase.
TU3A	209074_s_at	Hs00200376_m1	NM_007177	TU3A protein.
TYMS	202589_at	Hs00426591_m1	NM_001071	Thymidylate synthetase.
UBD	205890_s_at	Hs00197374_m1	NM_006398	Ubiquitin D.
UBE2C	202954_at	Hs00738962_m1	NM_181799	Ubiquitin-conjugating enzyme E2C.
UHRF1	225655_at	Hs00273589_m1	NM_013282	Ubiquitin-like containing PHD and RING finger domains 1.
URB	225242_s_at	Hs00736722_m1		Steroid sensitive gene 1

FIG. 8S

WFDC1	219478_at	Hs00221849_m1	NM_021197	WAP four-disulfide core domain 1.
WISP1	229802_at	Hs00365573_m1	2 RefSeqs	WNT1 inducible signaling pathway protein 1.
ZAK	225665_at	Hs00370447_m1	2 RefSeqs	Sterile alpha motif and leucine zipper containing kinase AZK.
ZBTB16	228854_at	Hs00232313_m1	NM_006006	zinc finger and BTB domain containing 16
ZFP36	201531_at	Hs00185658_m1	NM_003407	Zinc finger protein 36 C3H type homolog mouse.
ZNF217		Hs00232417_m1	NM_006526	Zinc finger protein 217.
ZWINT	204026_s_at	Hs00199952_m1		ZW10 interactor.
ZYX		Hs00170299_m1	NM_003461	Zyxin. Cell adhesion.
18S		Hs99999901_s1		Eukaryotic 18S rRNA

FIG. 8T

Gene Symbol	TaqMan Gene Expression Assay
ANK2	Hs00153998_m1
ANLN	Hs00218803_m1
ANXA10	Hs00200464_m1
APOBEC3B	Hs00358981_m1
ASAM	Hs00293345_m1
ASPM	Hs00411505_m1
BUB1B	Hs00176169_m1
C10orf3	Hs00216688_m1
C14orf78	Hs00746838_s1
CCNA2	Hs00153138_m1
CCNB1	Hs00259126_m1
CDC2	Hs00364293_m1
CDC20	Hs00415851_g1
CDCA1	Hs00230097_m1
CDCA3	Hs00229905_m1
CDKN3	Hs00193192_m1
CENPA	Hs00156455_m1
CENPF	Hs00193201_m1
CFH	Hs00164830_m1
ChGn	Hs00218054_m1
COL1A2	Hs00164099_m1
CRH	Hs00174941_m1
CTSE	Hs00157213_m1
CYP24A1	Hs00167999_m1

FIG. 9A

DLG7	Hs00207323_m1
EBF	Hs00395513_m1
F3	Hs00175225_m1
FABP6	Hs00155029_m1
FGFR3	Hs00179829_m1
FLJ11029	Hs00383634_m1
FLJ21986	Hs00227735_m1
FLJ31052	Hs00708284_s1
FN1	Hs00365058_m1
FOXM1	Hs00153543_m1
GJB2	Hs00269615_s1
GJB6	Hs00272726_s1
GUSB	Hs99999908_m1
HPRT	Hs99999909_m1
IGF2	Hs00171254_m1
INA	Hs00190771_m1
IQGAP3	Hs00603642_m1
KIAA0101	Hs00207134_m1
KIAA0186	Hs00221421_m1
KIAA0992	Hs00363101_m1
KIF11	Hs00189698_m1
KIF20A	Hs00194882_m1
KIF2C	Hs00199232_m1
KIF4A	Hs00602211_g1

FIG. 9B

Gene symbol	TaqMan Gene Expression Assay
KISS1	Hs00158486_m1
KLF9	Hs00230918_m1
KRT14	Hs00265033_m1
KRT20	Hs00300643_m1
KRT7	Hs00818825_m1
MAGEA3	Hs00366532_m1
MAGEA9	Hs00245619_s1
MAN1C1	Hs00220595_m1
MCM10	Hs00218560_m1
MELK	Hs00207681_m1
MKI67	Hs00606991_m1
MMP1	Hs00233958_m1
MMP12	Hs00159178_m1
NEK2	Hs00601227_mH
NFIA	Hs00325656_m1
NQO1	Hs00168547_m1
NR2F1	Hs00818842_m1
PDGFC	Hs00211916_m1
PDZRN3	Hs00392900_m1
PLAGL1	Hs00243030_m1
PLSCR4	Hs00220482_m1
POLQ	Hs00198196_m1
POSTN	Hs00170815_m1

FIG. 9C

PPIA o CYC	Hs99999904_m1
PPP1R12B	Hs00364078_m1
PPP1R14D	Hs00214613_m1
PRC1	Hs00187740_m1
PRKAR2B	Hs00176966_m1
PTPRC	Hs00236304_m1
RAMP	Hs00212788_m1
RNASE4	Hs00377763_m1
SBLF	Hs00538997_m1
SERPINB3	Hs00199468_m1
SLC1A6	Hs00192604_m1
SPON1	Hs00323883_m1
SPP1	Hs00167093_m1
STN2	Hs00263833_m1
TERT	Hs00162669_m1
TNRC9	Hs00300355_m1
TOP2A	Hs00172214_m1
TOPK	Hs00218544_m1
TPX2	Hs00201616_m1
TRIP13	Hs00188500_m1
TTK	Hs00177412_m1
UHRF1	Hs00273589_m1
ZBTB16	Hs00232313_m1
ZWINT	Hs00199952_m1
18S	Hs99999901_s1

FIG. 9D

Gene symbol	TaqMan Gene Expression Assay
ANLN	Hs00218803_m1
ANXA10	Hs00200464_m1
ASAM	Hs00293345_m1
ASPM	Hs00411505_m1
C14orf78	Hs00746838_s1
CCNA2	Hs00153138_m1
CDC2	Hs00364293_m1
CDC20	Hs00415851_g1
CDCA1	Hs00230097_m1
CENPF	Hs00193201_m1
CFH	Hs00164830_m1
CRH	Hs00174941_m1
CTSE	Hs00157213_m1
CYP24A1	Hs00167999_m1
EBF	Hs00395513_m1
FGFR3	Hs00179829_m1
FOXM1	Hs00153543_m1
GJB2	Hs00269615_s1
GUSB	Hs99999908_m1
IGF2	Hs00171254_m1
IQGAP3	Hs00603642_m1
KIF20A	Hs00194882_m1
KIF2C	Hs00199232_m1
KIF4A	Hs00602211_g1
KLF9	Hs00230918_m1

FIG. 10A

KRT14	Hs00265033_m1
KRT20	Hs00300643_m1
MAGEA3	Hs00366532_m1
MAGEA9	Hs00245619_s1
MCM10	Hs00218560_m1
MELK	Hs00207681_m1
MMP1	Hs00233958_m1
MMP12	Hs00159178_m1
NEK2	Hs00601227_mH
NR2F1	Hs00818842_m1
PDZRN3	Hs00392900_m1
POLQ	Hs00198196_m1
POSTN	Hs00170815_m1
PPIA	Hs99999904_m1
PPP1R14D	Hs00214613_m1
PTPRC	Hs00236304_m1
SLC1A6	Hs00192604_m1
TERT	Hs00162669_m1
TOP2A	Hs00172214_m1
TPX2	Hs00201616_m1
TRIP13	Hs00188500_m1
18S	Hs99999901_s1

FIG. 10B

BLADDER CANCER DIAGNOSIS AND/OR PROGNOSIS METHOD

This application is U.S. National Phase of International Application PCT/ES2007/000330, filed Jun. 5, 2007 designating the U.S., and published in a language other than English as WO 2008/113870 on Sep. 25, 2008, which claims priority to Spanish Patent Application No. P200700727, filed Mar. 20, 2007.

FIELD OF THE INVENTION

The field of application of the present invention is within the healthcare field, mainly in the "Oncological Urology" and "Molecular Biology" field. This invention is specifically aimed at bladder cancer diagnosis and prognosis methods.

BACKGROUND OF THE INVENTION

Bladder cancer, or vesical cancer, is the second most frequent tumor of the genitourinary tract after prostate cancer [Jemal A, Thomas A, Murray T, Thun M. *Cancer statistics,* 2002. *CA Cancer J Clin* 2002; 52:23-47]. In a global context, it represents approximately 3 and 1%, in men and women, respectively, of all the deaths due to cancer. In absolute values, this means that about 95,000 men and about 35,000 women die every year due to this pathology. The ratio between incidence and death is different depending on the degree of development of each country. As extreme examples, it could be mentioned that in the North America area this ratio would be close to 0.2, whereas in sub-Saharan regions it would increase up to 0.6 [Edwards B K, Brown M L, Wingo P A et al. *Annual report to the nation on the status of cancer,* 1975-2002*, featuring population-based trends in cancer treatment. J Natl Cancer Inst* 2005; 97:1407-27; Pisani P, Parkin D M, Bray F, Ferlay J. *Estimates of the worldwide mortality from* 25 *cancers in* 1990. *Int J Cancer* 1999; 83:18-29].

Unlike other tumors, familial genetic predisposition factors have virtually not been detected for the moment. In contrast, several environmental factors strongly related to bladder tumors have been detected. One of the most important factors, not only due to its relation to the disease but also due to its incidence in the population, is smoking. It has been observed that smokers have a risk three times higher than non-smokers of developing a bladder tumor. In fact, one third of bladder tumors are associated to tobacco consumption. Unfortunately, the carcinogenic agents present in tobacco have still not been clearly identified [Burch J D, Rohan T E, Howe G R et al. *Risk of bladder cancer by source and type of tobacco exposure: a case-control study. Int J Cancer* 1989; 44:622-28; Zeegers M P, Kellen E, Buntinx F, van den Brandt P A. *The association between smoking, beverage consumption, diet and bladder cancer: a systematic literature review. World J Urol* 2004; 21:392-401].

Different types of disorders can be found in the bladder at cell level. There are benign changes such as epithelial hyperplasias, urothelial metaplasias and Von Brunn's nests, among others. In contrast, dysplasias would correspond to disorders that are more or less intermediate between normal epithelium and carcinoma. Finally, different types of urothelial carcinomas are found in the bladder, which can divided into adenocarcinomas, squamous tumors and transitional cell carcinomas (TCC).

More than 90% of bladder tumors are TCCs. At the time of their diagnosis, approximately 75% are superficial tumors, 20% are invading muscular layers (infiltrating or invasive TCCs) and 5% are already metastatic. Of the superficial cases, approximately 20% are cured by means of a single surgical intervention, whereas between 50 and 70% recur one or more times after surgery, but never become infiltrating tumors. Between 10 and 30% of these superficial tumors become infiltrating tumors. These tumors are aggressive, poor-prognosis tumors with a mortality after 5 years of 50% and in the metastasized cases, the mortality after two years is 100% [Sanchez-Carbayo M, Socci N D, Charytonowicz E et al. *Molecular profiling of bladder cancer using cDNA microarrays: defining histogenesis and biological phenotypes. Cancer Res* 2002; 62:6973-80; Adshead J M, Kessling A M, Ogden C W. *Genetic initiation, progression and prognostic markers in transitional cell carcinoma of the bladder: a summary of the structural and transcriptional changes, and the role of developmental genes. Br J Urol* 1998; 82:503-12; Babaian R J, Johnson O F, Llamas L, Ayala A G. *Metastases from transitional cell carcinoma of urinary bladder. Urology* 1980; 16:142-44].

The genetic pathways of superficial and invasive TCCs, although related, seem to be quite different. The most usual progression in superficial tumors seems to be hyperplasia, atypia and finally low-grade papillary TCCs. In invasive tumors, it is most usual to progress from an atypia to a dysplasia, to then pass to a tumor in situ (Tis) and end in an infiltrating tumor [Knowles M A. *What we could do now: molecular pathology of bladder cancer. Mol Pathol* 2001; 54:215-21].

Current diagnosis systems are based on a combination of urinary cytology (from squamous cells in urine) and of the direct observation of the bladder by means of cystoscopy. The latter is actually the main diagnostic and follow-up technique for tumors. It is performed by transurethral route, therefore it is an invasive and rather unpleasant technique for the patients. The sensitivity and specificity of this technique were believed to be quite high, although improvements in the actual technique (fluorescence cystoscopy) indicate that this is probably not so and that part of the recurrence observed in superficial tumors could be due to the lack of total resection in non-visible parts thereof [Jones J S. *DNA-based molecular cytology for bladder cancer surveillance. Urology* 2006; 67:35-45]. Urinary cytology is in turn a non-invasive diagnostic technique with a high sensitivity and specificity for high-grade tumors. However, this technique shows limitations for detecting low-grade tumors [Bastacky S, Ibrahim S, Wilczynski S P, Murphy W M. *The accuracy of urinary cytology in daily practice. Cancer* 1999; 87:118-28]. Furthermore, the interpretation of the cytology is highly observer-dependent, therefore they may be inter-observer differences, especially in low-grade tumors.

All these limitations have led to the search for more reliable non-invasive bladder cancer markers. Finding a non-invasive marker with a high sensitivity and specificity for bladder TCC would be very helpful for clinical practice. In fact, several studies describe new tumor markers in urine, such as the test for the bladder tumor antigen NMP22 [Wiener H G, Mian C, Haitel A, Pycha A, Schatzl G, Marberger M. *Can urine bound diagnostic tests replace cystoscopy in the management of bladder cancer? J Urol* 1998; 159:1876-80; Soloway M S, Briggman V, Carpinito G A et al. *Use of a new tumor marker, urinary NMP*22*, in the detection of occult or rapidly recurring transitional cell carcinoma of the urinary tract following surgical treatment. J Urol* 1996; 156:363-67], fibrin degradation products [Schmetter B S, Habicht K K, Lamm D L et al. *A multicenter trial evaluation of the fibrin/fibrinogen degradation products test for detection and monitoring of bladder cancer. J Urol* 1997; 158:801-5.], telomerase [Takihana Y,

Tsuchida T, Fukasawa M, Araki I, Tanabe N, Takeda M. *Real-time quantitative analysis for human telomerase reverse transcriptase mRNA and human telomerase RNA component mRNA expressions as markers for clinicopathologic parameters in urinary bladder cancer. Int J Urol* 2006; 13:401-8], tests based on fluorescent in situ hybridization [Hailing K C, King W, Sokolova I A et al. *A comparison of BTA stat, hemoglobin dipstick, telomerase and Vysis UroVysion assays for the detection of urothelial carcinoma in urine. J Urol* 2002; 167:2001-6] or flow cytometry [Takahashi C, Miyagawa I, Kumano S, Oshimura M. *Detection of telomerase activity in prostate cancer by needle biopsy. Eur Urol* 1997; 32:494-98; Trott P A, Edwards L. *Comparison of bladder washings and urine cytology in the diagnosis of bladder cancer. J Urol* 1973; 110:664-66], but although most of them have a higher sensitivity than urinary cytology, the latter is still the most specific [Bassi P, De M, V, De Lisa A et al. *Non-invasive diagnostic tests for bladder cancer: a review of the literature. Urol Int* 2005; 75:193-200].

It is known that many and very varied genetic disorders are found in urothelial tumors, therefore the current tendency is to search for genetic markers (either at the DNA, RNA or protein level) which can indicate the presence of carcinomas in the analyzed sample. Furthermore, it would be very interesting to be able to discriminate the aggressiveness of the tumor of a patient with these same markers, as this would allow a much more personalized and effective treatment. Finally, some of these markers could be possible therapeutic targets for developing new drugs to combat cancer.

Until recently, the capacity to analyze gene expression patterns was limited to a few genes per experiment. New technologies, such as DNA microarrays have completely changed the scenario. Thousands of genes can currently be analyzed in a single assay [Duggan D J, Bittner M, Chen Y, Meltzer P, Trent J M. *Expression profiling using cDNA microarrays. Nat Genet* 1999; 21:10-14; Granjeaud S, Bertucci F, Jordan B R. *Expression profiling: DNA arrays in many guises. Bioessays* 1999; 21:781-90]. Therefore, massive expression results of all tumor types have started to appear in literature, including bladder tumors [Sanchez-Carbayo M, Socci N D, Charytonowicz E et al. *Molecular profiling of bladder cancer using cDNA microarrays: defining histogenesis and biological phenotypes. Cancer Res* 2002; 62:6973-80; Ramaswamy S, Tamayo P, Rifkin R et al. *Multiclass cancer diagnosis using tumor gene expression signatures. Proc Natl Acad Sci USA* 2001.98:15149-54; Sanchez-Carbayo M, Socci N D, Lozano J J et al. *Gene discovery in bladder cancer progression using cDNA microarrays. Am J Pathol* 2003; 163:505-16; Sanchez-Carbayo M, Capodieci P, Cordon-Cardo C. *Tumor suppressor role of KiSS-1 in bladder cancer loss of KiSS-1 expression is associated with bladder cancer progression and clinical outcome. Am J Pathol* 2003; 162:609-17; Dyrskjot L, Thykjaer T, Kruhoffer M et al. *Identifying distinct classes of bladder carcinoma using microarrays. Nat Genet* 2003; 33:90-96], although most of the results have not been made public in their entirety. However, up until now, the studies which have been conducted with specific bladder cancer markers have been focused on one or on very few genes [Olsburgh J, Hamden P, Weeks R et al. *Uroplakin gene expression in normal human tissues and locally advanced bladder cancer. J Pathol* 2003; 199:41-49; Fichera E, Liang S, Xu Z, Guo N, Mineo R, Fujita-Yamaguchi Y. A quantitative reverse transcription and polymerase chain reaction assay for human IGF-II allows direct comparison of IGF-II mRNA levels in cancerous breast, bladder, and prostate tissues. Growth Horm IGF Res 2000; 10:61-70; Simoneau M, Aboulkassim T O, LaRue H, Rousseau F, Fradet Y. *Four tumor suppressor loci on chromosome 9q in bladder cancer: evidence for two novel candidate regions at* 9q22.3 *and* 9q31. *Oncogene* 1999; 18:157-63].

Given that the nature of these tumors is very heterogeneous, it does not seem very likely to be able to identify all or most carcinomas with a single marker. Thus, to be able to characterize most tumors it seems to be essential to combine several of the best markers to some type of extent.

In addition, although the direct analysis of urothelial tissue is the most comfortable alternative for developing a routine diagnostic method, it would be very interesting, as has been mentioned above, that said method were not invasive, because the latter decrease the quality of life of the patients and represent a much higher economic burden for healthcare.

Bladder fluids (urine or bladder washing) which are in contact with the entire bladder epithelium, and therefore with the tumor mass, seem to be a good alternative for detecting tumor markers, given that they represent an easy and non-invasive way to obtain the sample to be analyzed. Thus, a large number of works have been focused on the study of tumor markers in urine in the search for a non-invasive diagnostic method for bladder TCC. In fact, different tests with this objective have been marketed (NMP22, UroVysion, ImmunoCyt, Accu-Dx, etc.).

One alternative, which has still not been marketed, is the detection of bladder TCC in urine samples by means of determining the gene expression of bladder cancer markers. In fact, there are some studies suggesting the usefulness of this methodology, although they have been conducted with one or a few marker genes [Parekattil S J, Fisher H A, Kogan B A. *Neural network using combined urine nuclear matrix protein-22, monocyte chemoattractant protein-1 and urinary intercellular adhesion molecule-1 to detect bladder cancer. J Urol* 2003; 169:917-20; Eissa S, Kenawy G, Swellam M, El Fadle A A, Abd El-Aal A A, El Ahmady O. *Comparison of cytokeratin* 20 *RNA and angiogenin in voided urine samples as diagnostic tools for bladder carcinoma. Clin Biochem* 2004; 37:803-10; Larsson P C, Beheshti B, Sampson H A, Jewett M A, Shipman R. *Allelic deletion fingerprinting of urine cell sediments in bladder cancer. Mol Diagn* 2001; 6:181-88].

In response to these needs, the inventors, after an important research work, have identified 14 bladder tumor marker genes, from which they have developed a bladder cancer diagnosis and prognosis method based on the detection and quantification of the gene expression of these genes by means of quantitative real-time PCR in RNA extracted from bladder fluids, and their subsequent computer combination by means of an "alarm system".

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** (A-J): Electropherograms obtained with the Agilent 2100 Bioanalyzer of the samples of intact RNA (BW0) (FIG. **1**.A) and partially degraded RNA (BW1, BW2, BW3) (FIGS. **1**.B, **1**.C, **1**.D) of bladder washing, bladder tumor (T0, T1, T2, T3) (FIGS. **1**.E, **1**.F, **1**.G, **1**.H) and pool of control samples (C) (FIG. **1**.**1**) and gel with the bands of the ribosomal RNAs (28S and 18S) of each of the analyzed samples (FIG. **1**.J). The numbers 0, 1, 2 and 3 are assigned to the samples in increasing order of degradation and for the samples with RNA of a comparable quality.

FIG. **2**: (a) Semi-matrix of comparison between pairs of arrays of the 100 most differentially expressed genes in each array. The upper and lower part shaded in gray of the semi-matrix shows the percentage of differentially expressed genes in common between the pairs of bladder washing (BW) arrays

and between the pairs of tumors (T) arrays, hybridized with RNA of a different degree of degradation. The non-shaded part of the semi-matrix corresponds to the percentage of differentially expressed genes in common between the pairs of bladder washing and tumor arrays. (b) Unsupervised cluster of all the clones contained in the microarray, including the duplicates with dye swap (DS).

FIG. **3**: Validation by means of quantitative real-time RT-PCR (qRT-PCR) of 4 differentially expressed genes (KRT20, GSN, IGF2 and CCL2) in the cDNA microarrays and related to bladder cancer, in 36 additional tumor bladder washing samples. The positive values indicate overexpression in the tumor bladder washings in relation to the controls. The samples are grouped in the graph depending on the log 2ratio according to the tumor stage and grade: low-grade superficial tumors (8 pTa and 3 pT1), high-grade superficial tumors (5 pTa, 5 pT1 and 4 pTis) and invasive tumors (9 pT2 and 2 pT4).

FIG. **4**: Classification of the samples by means of unsupervised global cluster (Euclidean distance and UPGMA). pT2_1, pT2_2 and pT2_3 (infiltrating tumors); pT1 HG_1, pT1 HG_2 and pT1 HG_3 (high-grade superficial tumors); pT1 LG_1, pT1 LG_2, pT1 LG_3 (low-grade superficial tumors).

FIG. **5**: Results of quantitative real-time PCR (qRT-PCR) of the pools and the individual samples contained therein. The table is divided into pools (left part) and into individual samples (right). The columns of the pools indicate the pool number (No.), the expression levels observed in the microarrays (μarrays) and the levels quantified by means of qRT-PCR (qRT-PCR). The first column of the individual samples corresponds to the arithmetic mean of the expressions of the individual samples contained in the pool (mean), which are indicated in the following columns (1-5). Each row corresponds to a gene (TCN1, SORBS1, MYH11, SRPX, CRH, KRT14, RRM2, FOSB, CEACAM6, CES1), with the different expression levels for each pool.

FIG. **6**: Classification of the 60 individual bladder fluid samples by means of an unsupervised global cluster of 384 genes (Euclidean distance and UPGMA). The nomenclature of the samples follows the following rules: if the sample starts with the letter "B", it refers to a tumor bladder washing sample; if it starts by "CB", it refers to a control bladder fluid sample; if the initials "RV" appear after the sample number it refers to a bladder washing sample; if on the other hand, the initial "O" appears, it refers to a urine sample, and the tumor grade and pathological condition of the tumor sample is indicated after the underscore. The arrows indicate a bad classification of the sample which they indicate in relation to the established categories: T_HG (high-grade tumors); T_LG (low-grade tumors), and C (controls).

FIGS. **7** (A and B): Classification of the 140 individual bladder fluid samples by means of an unsupervised global cluster of 96 genes (Euclidean distance and UPGMA). The arrows indicate a bad classification of the sample which they indicate in relation to the established categories (C, controls; FIG. **7**.A and T, tumor samples; FIG. **7**.B). The nomenclature of the samples follows the following rules: if the sample starts with the letter "B", it refers to a tumor bladder fluid sample; if it starts by "CB", it refers to a control bladder fluid sample; if the initial "R" appears after the sample number, it refers to a bladder washing sample; if on the other hand, the initial "O" appears, it refers to a urine sample.

FIG. **8** (A-T): List of 384 diagnostic, prognostic and endogenous control genes for bladder cancer. This list has been obtained from the analysis by means of Affymetrix microarrays of pools of bladder tumor tissue samples with different stages and tumor grades and control bladder mucosa samples.

FIG. **9** (A-D): List of 96 diagnostic, prognostic and endogenous control genes for bladder cancer. This list has been obtained by means of the analysis of 60 bladder fluid samples in microfluidic cards containing the 384 genes of FIG. **8**. The gene symbol and the name of the TaqMan Gene Expression Assay selected for the TaqMan Low Density Array microfluidic card are indicated.

FIGS. **10** (A and B): List of 48 diagnostic, prognostic and endogenous control genes for bladder cancer. This list has been obtained by means of the analysis of 140 bladder fluid samples in microfluidic cards containing the 96 genes of FIG. **9**. The gene symbol and the name of the TaqMan Gene Expression Assay selected for the TaqMan Low Density Array microfluidic card are indicated.

OBJECT OF THE INVENTION

The object of the present invention relates to an in vitro non-invasive bladder cancer diagnosis and/or prognosis method based on the detection and quantification in bladder fluids of the gene expression of certain genes and/or combinations thereof acting as genetic markers of said disease.

Likewise, the use of said genes as bladder cancer diagnosis and/or prognosis genetic markers is an object of the present invention.

Finally, another object of the invention relates to a bladder cancer diagnosis and/or prognosis kit based on the use of said genes as genetic markers of the disease.

DESCRIPTION OF THE INVENTION

The main objective of the present invention is to develop an in vitro non-invasive bladder cancer diagnosis and/or prognosis method based on the detection and quantification of certain genes acting as genetic markers of the disease.

To carry out the method, the starting point is a bladder fluid sample obtained from a subject on which an analysis is conducted for the detection and quantification of the expression pattern of certain genes and/or combinations thereof. The results obtained are compared with the normal reference values for said genes in bladder fluids to thus establish the diagnosis and/or prognosis.

The term "subject" used in the present invention relates to a human being.

The bladder fluid sample obtained from the subject can be a urine or bladder washing sample and can be obtained by means of any conventional method.

In the present invention, bladder cancer diagnosis method is understood as that which allows detecting and quantifying differentially expressed genes between tumors and control samples (from healthy individuals) (diagnostic genes).

The prognosis method relates to those which allow detecting differentially expressed genes in the different types of tumors (prognostic genes), which allows classifying the tumors according to aggressiveness and personalizing the treatment in each case.

The tumor classification of the different types of transitional cell carcinomas (TCCs) is currently based on the macroscopic and microscopic observation in the pathological anatomy laboratory. Their classification is decided by means of more or less standardized observations, based on the depth of the tumor and on the microscopic appearance of the cells. Recent molecular studies seem to indicate that there are actu-

ally two differential genetic profiles which mostly separate superficial type tumors and infiltrating tumors.

Superficial bladder tumors are thus called Ta, Tis and T1. The Ta carcinoma is an exophytic carcinoma that is non-invasive or confined to the epithelium. Tis is a carcinoma in situ (flat superficial tumor) and T1 is a tumor invading the subepithelial connective tissue or invading the lamina propria.

In the present invention, the abbreviation HG is used to determine high-grade tumors and LG to determine low-grade tumors.

The Ta and T1 carcinomas can be extirpated by means of transurethral resection (TUR). Although high-grade (HG) Tis and T1 are superficial carcinomas confined to the mucosa, because they are high-grade tumors, it has been demonstrated with molecular biology techniques and by clinical experience that they have a high malignant and invasion potential.

In addition, infiltrating bladder carcinomas are classified into T2, T3 and T4. Thus, T2 relates to a tumor invading the muscular bladder layer. This type is in turn divided into T2a, invading the superficial muscular layer or the inner half, and T2b, invading the deep muscular layer or the outer half. T3 relates to a tumor invading beyond the muscular layer or invading the perivesical fat. This type is in turn divided into T3a, with microscopic invasion, and T3b, with macroscopic invasion. Finally, T4 relates to a tumor invading structures adjacent to the urinary bladder and which is in turn divided into T4a, with prostate, uterus or vagina invasion, and T4b, with pelvic wall or abdominal wall invasion.

The detection and quantification of the gene expression of the genes can be carried out by means of any non-invasive molecular biology technique suitable for the purposes of the invention, such as for example expression microarrays, quantitative real-time PCR, northern blot, conventional PCR, etc.

Specifically, the use of DNA arrays allows obtaining expression results of a very high number of genes, allowing to test thousands of genes in each experiment. The use of this technique requires large amounts of RNA with a good quality (non-degraded).

The quantitative real-time PCR technique (qRT-PCR) is preferably used in the present invention to detect and quantify the diagnostic and/or prognostic genes. This technique is more accurate, in addition to allowing the use of RNA with a considerable degree of degradation, without this affecting the end result. Likewise, it allows quantifying the specific RNA of the genes of interest. In particular embodiments, the hybridization probes used are Taqman probes.

The results obtained in the detection and quantification of the expression of the genes in the bladder fluid sample are compared with the normal reference values for said genes in samples from healthy subjects. The increase or the decrease of the marker genes levels are generally estimated by means of comparing the results obtained from the analysis of the samples corresponding to the subjects of the assay with the results of the control samples, which are analyzed in parallel. The final decision of the classification of each sample is made by means of an "alarm system" based on the expression values of the marker genes, such that if any of the values observed shows a very significant deviation in relation to what is expected in a control sample, the probability that a final classification is a tumor classification greatly increases, regardless of the gene which has "given the alarm".

More specifically, in a main aspect of the invention, the non-invasive bladder cancer diagnosis and/or prognosis method comprises collecting a bladder fluid sample from a subject to carry out the detection and quantification in said sample of the expression pattern of the combination of ANXA10, C14orf78, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6, TERT, ASAM and MCM10 genes. The results obtained were compared to the normal reference values for said genes in bladder fluids.

The bladder fluid sample is preferably urine, given that it is obtained much more easily. Nevertheless, bladder washing is occasionally done in a routine manner and RNA with a better quality is obtained

The ANXA 10 (annexin A10) gene (also called ANX14), located in 4q32.3, participates in cell division regulation and in different signal transduction pathways, but their exact function has still not been determined.

The C14orf78 (chromosome 14 open reading frame 78) gene (also called AHNAK2 or KIAA2019) is located in 14q32.33. Its function has still not been determined.

The CTSE (cathepsin E) gene (also called CATE), located in 1q31, encodes an intracellular protease.

The CRH (corticotropin releasing hormone) gene (also called CRF), located in 8q13, encodes the corticotropin releasing hormone, secreted in the hypothalamus in response to stress.

The IGF2 (insulin-like growth factor 2 (somatomedin A)) gene (also called 11orf43, FLJ22066, FLJ44734, INSIGF), located in 11p15.5, encodes the insulin-like growth factor.

The KLF9 (Kruppel-like factor 9) gene (also called BTEB1) encodes a transcription factor.

The KRT20 (Keratin 20) gene (also called K20; CK20, KRT21; MGC35423), located in 17q21.2, encodes a protein forming part of the intermediate filaments in charge of giving structure and integrity to epithelial cells.

The MAGEA3 (melanoma antigen family A, 3) gene (also called HIP8; HYPD; MAGE3; MAGEA6; MGC14613) is located in Xq28. Its function is unknown.

The POSTN (periostin, osteoblast specific factor) gene (also called PN; OSF-2; PDLPOSTN; MGC119510, MGC119511, periostin; RP11-412K4.1) is located in 13q13.3 and has a function related to cell mobility.

The PPP1R14D (protein phosphatase 1, regulatory (inhibitor) subunit 14D) gene (also called GBPI-1; FLJ20251; MGC119014, MGC119016, CPI17-like) is located in 15q15.1 and encodes a phosphatase.

The SLC1A6 (solute carrier family 1 (high affinity aspartate/glutamate transporter), member 6) gene (also called EAAT4; MGC33092, MGC43671), located in 19p13.12, participates in intracellular transport.

The TERT (telomerase reverse transcriptase) gene (also called TP2; TRT; EST2; TCS1, hEST2), located in 5p15.33, encodes a polymerase of telomeres with reverse transcriptase activity.

The ASAM (adipocyte-specific adhesion molecule) gene (also called ASAM; ACAM; FLJ22415), located in 11q24.1, participates in cell adhesion.

Finally, the MCM10 (minichromosome maintenance deficient 10 (S. cerevisiae)) gene (also called CNA43; PRO2249, MGC126776), located in 10p13, encodes a protein involved in the initiation of genomic replication.

In another aspect of the invention, the in vitro non-invasive bladder cancer diagnosis and/or prognosis method comprises collecting a bladder fluid sample from a subject to carry out the detection and quantification in said sample of the expression pattern of the combination of ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6, TERT, and MCM10 genes. The results obtained are compared with the normal reference values for said genes in bladder fluids.

In another aspect of the invention, the in vitro non-invasive bladder cancer diagnosis and/or prognosis method comprises collecting a bladder fluid sample from a subject to carry out

the detection and quantification in said bladder fluid sample of the expression of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D, ASAM and combinations thereof. The results obtained are compared with the normal reference values for said genes in bladder fluids.

Thus, in one particular aspect of the invention, said diagnosis and/or prognosis method based on the individual detection and quantification of the expression of the C14orf78 gene is contemplated.

In another particular aspect of the invention, said diagnosis and/or prognosis method based on the individual detection and quantification of the expression of the KLF9 gene is contemplated.

In another particular aspect of the invention, said diagnosis and/or prognosis method based on the individual detection and quantification of the expression of the POSTN gene is contemplated.

In another particular aspect, said diagnosis and/or prognosis method based on the individual detection and quantification of the expression of the PPPIR14D gene is contemplated.

In another particular aspect of the invention, said diagnosis and/or prognosis method based on the individual detection and quantification of the expression of the ASAM gene is contemplated.

In another particular embodiment of the invention, an in vitro non-invasive bladder cancer diagnosis and/or prognosis method based on the detection and quantification of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D, ASAM and combinations thereof and, additionally, at least one gene selected from ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6, TERT and MCM10 is contemplated.

In another aspect of the invention, an in vitro non-invasive method focused on bladder cancer diagnosis is contemplated which comprises collecting a bladder fluid sample from a subject to carry out the detection and quantification of the expression pattern of the combination of ANXA10, C14orf78, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6 and TERT genes, according to the previously described method. The results obtained are compared with the normal reference values for said genes in bladder fluids.

In another aspect of the invention, the in vitro non-invasive bladder cancer diagnosis method comprises collecting a bladder fluid sample from a subject to carry out the detection and quantification of the expression pattern of the combination of ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6 and TERT genes. The results obtained are compared with the normal reference values for said genes in bladder fluids.

In another aspect of the invention, the in vitro non-invasive bladder cancer diagnosis method comprises collecting a bladder fluid sample from a subject to carry out the detection and quantification of the expression of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D and combinations thereof. The results obtained are compared with the normal reference values for said genes in bladder fluids.

In a particular embodiment of the invention, said diagnosis method is based on the detection and quantification of the C14orf78 gene.

In another particular embodiment of the invention, said diagnosis method is based on the detection and quantification of the KLF9 gene.

In another particular embodiment of the invention, said diagnosis method is based on the detection and quantification of the POSTN gene.

In another particular embodiment of the invention, said diagnosis method is based on the detection and quantification of the PPPIR14D gene.

In another particular embodiment of the invention, said diagnosis method is based on the detection and quantification of the expression of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D and combinations thereof and, additionally, at least one gene selected from ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6 and TERT.

In another aspect of the invention, an in vitro non-invasive bladder cancer prognosis method is contemplated which comprises collecting a bladder fluid sample from a subject to carry out the detection and quantification of the expression pattern of the combination of ASAM and MCM10 genes. The results obtained are compared with the normal reference values for said genes in bladder fluids.

Another aspect of the invention provides an in vitro non-invasive bladder cancer prognosis method which comprises collecting a bladder fluid sample from a subject to carry out the detection and quantification of the expression of the ASAM gene. The results obtained are compared with the normal reference values for said gene in bladder fluids.

In another aspect of the invention, the use of the combination of ANXA10, C14orf78, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6, TERT, ASAM and MCM10 genes as bladder cancer diagnosis and/or prognosis markers is contemplated.

Another aspect of the invention is focused on the use of the combination of ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6, TERT, and MCM10 genes as bladder cancer diagnosis and/or prognosis markers.

In another aspect of the invention, the use of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D, ASAM and combinations thereof as bladder cancer diagnosis and/or prognosis markers is contemplated.

In a particular embodiment of the invention, the use of the C14orf78 gene as a bladder cancer diagnosis and/or prognosis marker is contemplated.

In another particular embodiment of the invention, the use of the KLF9 gene as a bladder cancer diagnosis and/or prognosis marker is contemplated.

In another particular embodiment of the invention, the use of the POSTN gene as a bladder cancer diagnosis and/or prognosis marker is contemplated.

In another particular embodiment of the invention, the use of the PPP1R14D gene as bladder cancer diagnosis and/or prognosis markers is contemplated.

In another particular embodiment of the invention, the use of the ASAM gene as a bladder cancer diagnosis and/or prognosis marker is contemplated.

Another aspect of the invention is focused on the use of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D, ASAM and combinations thereof, in combination with at least one gene selected from ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6, TERT and MCM10, as bladder cancer diagnosis and/or prognosis markers.

Another aspect of the invention relates to the use of the combination of ANXA10, C14orf78, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6 and TERT genes as bladder cancer diagnosis markers.

Another aspect of the invention is focused on the use of the combination of ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6 and TERT genes as bladder cancer diagnosis markers.

Another aspect of the invention relates to the use of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D and combinations thereof as bladder cancer diagnosis markers.

In a particular embodiment of the invention, the use of the C14orf78 gene as a bladder cancer diagnosis marker is contemplated.

Likewise, in another particular embodiment of the invention, the use of the KLF9 gene as a bladder cancer diagnosis marker is contemplated.

In another particular embodiment of the invention, the use of the POSTN gene as a bladder cancer diagnosis marker is contemplated.

In another particular embodiment of the invention, the use of the PPP1R14D gene as bladder cancer diagnosis markers is contemplated.

In another aspect of the invention, the use of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D and combinations thereof, in combination with at least one gene selected from ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6 and TERT, as bladder cancer diagnosis markers is contemplated.

In another aspect of the invention, the use of the combination of ASAM and MCM10 genes as bladder cancer prognosis markers is contemplated.

In another aspect of the invention, the use of the ASAM gene as a bladder cancer prognosis marker is contemplated.

Another aspect of the invention relates to a bladder cancer diagnosis and/or prognosis kit comprising a set of probes suitable for the detection and quantification of the expression pattern of the combination of ANXA10, C14orf78, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6, TERT, ASAM and MCM10 genes.

In another aspect of the invention, the bladder cancer diagnosis and/or prognosis kit comprises a set of probes suitable for the detection and quantification of the expression pattern of the combination of ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6, TERT and MCM10 genes.

In another aspect of the invention, a bladder cancer diagnosis and/or prognosis kit based on a set of probes suitable for the detection and quantification of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D, ASAM and combinations thereof is contemplated.

In a particular embodiment of the invention, said bladder cancer diagnosis and/or prognosis kit, based on the set of probes for the detection and quantification of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D, ASAM and combinations thereof, additionally comprises a set of probes suitable for the detection and quantification of a gene selected from ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6, TERT and MCM10.

In another aspect of the invention, a bladder cancer diagnosis kit, based on a set of probes suitable for the detection and quantification of the expression pattern of the combination of ANXA10, C14orf78, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6 and TERT genes, is contemplated.

In another aspect of the invention, the bladder cancer diagnosis kit comprises a set of probes suitable for the detection and quantification of the expression pattern of the combination of ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6 and TERT genes.

In another aspect of the invention, the cancer diagnosis kit based on a set of probes suitable for the detection and quantification of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D and combinations thereof is contemplated.

In another aspect of the invention, said kit, based on the set of probes suitable for the detection and quantification of a gene selected from C14orf78, KLF9, POSTN, PPP1R14D and combinations thereof, additionally comprises probes suitable for the detection and quantification of at least one gene selected from ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6 and TERT.

In another aspect of the invention, a bladder cancer prognosis kit, based on a set of probes suitable for the detection and quantification of the expression pattern of the combination of ASAM and MCM10 genes, is contemplated.

In another aspect of the invention, the prognosis kit is based on a probe suitable for the detection and quantification of the ASAM gene.

Table 1 shows the 14 genes identified as bladder cancer diagnosis and/or prognosis genetic markers. The ASAM and MCM10 genes are the 2 specific genes for prognosis.

Several examples which are useful for illustrating but not for limiting the present invention are set forth below.

EXAMPLES

Example 1

Determination of the Importance of Degradation in Bladder Fluid Samples

To carry out the final objective of the invention, it was first necessary to know the impact of different RNA degradation levels on gene expression profiles, given that the quality of the RNA obtained from bladder fluids (urine and/or bladder washing) is generally low. It also had to be determined if the gene expression profiles obtained from the bladder fluids matched those obtained in the corresponding tumors.

1. Selection of Samples and RNA Preparation

A tumor tissue (T) and bladder washing (BW) sample from one and the same patient diagnosed as high-grade (G3) pT2 was selected [according to the methods described in Lopez-Beltran A, Sauter G, Gasser T, Hartmann A, Schmitz-Drager B J, Helpap B, Ayala A G, Tamboni P, Knowles M A, Sidransky D, Cordon-Cardo C, Jones P A, Cairns P, Simon R, Amin M B, Tyczynsky J E. Tumours of the Urinary System. In: Eble J N, Sauter G, Epstein J I, Sesterhenn I A (eds.), *Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. World Health Organization Classification of Tumours. Lyon: IARC Press;* 2004: 89-157; Sobin L H, Witekind C H. *TNM Classification of Malignant Tumours. International Union Against Cancer.,* 6th ed. New York: John Wiley & Sons; 2002]. The RNA of both samples (T0 and BW0) was extracted with TRIzol (Invitrogen, Carlsbad, Calif., USA) according to the supplier's instructions. Aliquots of both RNAs (T0 and BW0) were then degraded by incubating them at 80° C. for 15 (T1 and BW1), 30 (T2 and BW2) and 60 (T3 and BW3) minutes, obtaining three degradation levels, as described in Xiang C C, Chen M, Ma L et al. *A new strategy to amplify degraded RNA from small tissue samples for microarray studies. Nucleic Acids Res* 2003; 31:53, with the exception that water was used instead of a basic buffer.

Healthy bladder mucosa samples from 4 patients without evidence of bladder pathology (control samples) were also collected, RNA was obtained in the same manner as with the previous samples and the 4 RNAs were mixed in equimolar ratios (C0).

One μl of each of the intact and degraded RNAs were analyzed in the Agilent 2100 Bioanalyzer to determine the quality of each RNA (according to the method described in Imbeaud S, Graudens E, Boulanger V et al. *Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces. Nucleic Acids Res* 2005; 33:e56) (FIGS. **1**.A-H). FIG. **1** (J) shows the gel with the bands of the ribosomal RNAs (28S and 18S) of each of the analyzed samples in which the progressive degradation of these bands is observed.

In addition, 36 tumor bladder washings (8 low-grade (LG) pTa, 5 high-grade (HG) pTa, 3 pT1 LG, 5 pT1 HG, 4 pTis, 9 pT2 HG and 2 pT4 HG) and 14 control bladder washings from patients without bladder pathology were collected and the RNA was extracted in the same manner as in the previous cases.

2. In vitro RNA Amplification and Labeling

5 μg of intact RNA (T0 and BW0) and degraded RNA (T1, T2, T3, BW1, BW2 and BW3) were amplified by means of using primers with a random sequence of 9 nucleotides (random nonamer primers) modified by the addition in 3' of the T3 promoter sequence (T3N9) (according to Xiang C C, Chen M, Ma L et al. *A new strategy to amplify degraded RNA from small tissue samples for microarray studies. Nucleic Acids Res* 2003; 31:e53). The probes were synthesized by means of a direct labeling method (according to Richter A, Schwager C, Hentze S, Ansorge W, Hentze M W, Muckenthaler M. *Comparison of fluorescent tag DNA labeling methods used for expression analysis by DNA microarrays. Biotechniques* 2002; 33:620-8, 630).

3. Array Processing and Data Analysis

Oncochip-v2 glass human cDNA microarrays were used to co-hybridize each of the four progressively degraded aliquots of RNA, both of tumor and of bladder washing (T0, T1, T2, T3, BW0, BW1, BW2 and BW3), with the pool of the RNAs from healthy bladder mucosa samples (C0). The fluorescent images were obtained with the G2565BA Microarray Scanner System (Agilent, Technologies, Waldbronn, Germany) and the TIFF images were quantified using the Spot program which is available from the Commonwealth Scientific and Industrial Research Organization (CSIRO) of Australia under the R statistical environment which is available from the database hosted by the Institute for Statistics and Mathematics of Wirtschaftsuniversität Wien. The final intensity measurement of each point of the microarray was calculated as had been previously suggested in the Microarray DATA Analysis Group managed by "Speed Berkeley Research Group", University of Berkeley, Berkeley, Caliif. (publicly accessible data in the GEO database; GSE3192). Finally, 1111 valid clones were obtained which complied with all the quality criteria and the 100 most differentially expressed clones of each array were chosen to compare between arrays and calculate the percentages of genes in common between them. A high percentage of differentially expressed genes in common was detected between the tumor tissue arrays (85 to 91%) and between the bladder washing arrays (78 to 93%) (FIG. **2***a*), which indicated that RNA degradation virtually did not affect the gene expression profiles.

An unsupervised cluster of all the clones contained in the microarray was also carried out using UPGMA (Unweighted Pair Group Method with Arithmetic mean) and Pearson's correlation. This cluster indicated that the percentage of genes in common identified between 2 arrays hybridized with RNA with a different degradation level (for example, BW0 and BW1) was occasionally higher than the percentage between dye swap (DS) duplicates of the same array (for example, BW0 vs. BW0-DS) (FIG. **2***b*), which reinforced the conclusion that the gene expression profiles were virtually not altered when working with partially degraded RNA.

To determine if the gene expression profiles obtained from the bladder washing samples matched those obtained in the corresponding tumor, the percentage of differentially expressed genes in common between the tumor tissue arrays and the bladder washing arrays was compared. A high similarity between the tumor and the bladder washing was obtained (52 to 60%) and this similarity was independent of the RNA degradation condition.

In conclusion, this data suggested that partially degraded bladder washing RNA could be used for gene expression studies using microarrays and that this RNA is a reflection of the gene expression of the tumor.

4. Quantitative Real-time RT-PCR (qRT-PCR)

To validate that the results obtained in the microarrays of a particular patient could be extrapolated to a longer cohort, 4 differentially expressed genes in the arrays which were related to the bladder carcinogenesis process according to the literature (KRT20, IGF2, GSN and CCL2) were analyzed by means of qRT-PCR. For this validation, 36 additional tumor bladder washings and 14 control bladder washings were used.

The cDNA was synthesized from 1 ug of RNA using the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, USA) according to the supplier's instructions, except that the final volume of the reaction was reduced to 50 μl. The GUSB gene was used as an endogenous control. The PCRs were carried out using Assays-on-Demand™ Gene Expression Products in an ABI PRISM 7000 SDS (Applied Biosystems, Foster City, USA) according to the supplier's instructions, except that the volume of the reaction was reduced to 20 μl.

The ΔΔCt method (ABI PRISM 7700 Sequence Detection System User Bulletin #2: Relative Quantification of Gene Expression P/N 4303859) was used to calculate the relative amount of expression of each gene in relation to an average of the expression of the 14 control samples. To establish the reference value in the controls, the arithmetic mean of the expression values of the 14 control bladder washings from patients without bladder pathology was obtained.

The results of this analysis, expressed as log 2ratio, defined as the proportion or division (ratio) between the 2 compared conditions (in this case the endogenous control against each gene indicated) expressed as a base 2 logarithm, confirmed the results of the microarrays in 81% of the samples for KRT20, in 89% for GNS, in 64% for IGF2 and in 89% for CCL2 (FIG. **3**). The high consistency between the microarray data obtained from the analysis of a single patient and the qRT-PCR data obtained from the analysis of a cohort of 36 additional patients confirmed that the gene expression profiles obtained in the microarrays were not due to the analysis of a single patient.

5. Conclusion of Example 1

It is possible to use bladder washing RNA to deduce the gene expression profiles of the corresponding bladder tumors, both by means of cDNA microarrays and of qRT-PCR.

Example 2

Initial Determination of Candidate Genes for the Predictive Model

Once it was known that it was possible to determine gene expression profiles in bladder fluid samples, the next objective was to obtain characteristic gene expression data that was as extensive as possible. The decision was made to follow the strategy of starting by analyzing the largest possible amount of genes in a reduced number of samples, in order to progressively analyze an increasingly smaller and more selected amount of genes in a more extensive series of samples in successive phases.

The protocol in turn involved the establishment of very strict quality controls in all the critical steps of the process. This included obtaining biological samples with the desired characteristics in the operating room by surgeons of the Fundació Puigvert team, storing and preserving the samples in the

suitable conditions, the anatomical-pathological analysis of the samples and the molecular processing by the laboratory equipment.

1. Obtaining and Selecting the Biological Samples

The tissue samples were obtained in the operating room using a cold forceps resector (tumor samples) or directly with scissors (control samples). Part of the tissue obtained was immediately frozen at −80° C. until being subsequently processed for RNA extraction and remaining part was sent to the Pathological Anatomy department for its anatomical-pathological analysis. For the RNA extraction, the tissues were mechanically homogenized and the RNAs were extracted according to the protocol of TRIzol (Invitrogen, Carlsbad, Calif., USA). Finally, the RNAs were quantified by spectrophotometrically measuring the absorbance at 260 nm.

2. Groups of Samples to be Studied

Given that superficial tumors and invasive tumors seem to have different genetic profiles, the decision was made to compare the most extreme tumor groups (low-grade superficial tumors versus infiltrating tumors). Furthermore, the decision was also made to find out the molecular profile of a type of tumor with an unclear clinical behavior, because they are tumors that are superficial but have a high degree of cell aberrations (classified as high-grade T1, pT1 HG) and in many case (about 50%) end up being infiltrating tumors. Four study groups were thus defined:

- Group 1; low-grade (LG) superficial tumor samples which only invade the bladder mucosa (pathologically classified as pTa LG).
- Group 2: high-grade (HG) superficial tumor samples which invade the subepithelial connective tissue (pathologically classified as pT1 HG).
- Group 3: infiltrating and high-grade tumor samples (pathologically classified as pT2).
- Group 4: healthy bladder mucosa samples (control).

For the purpose of reducing the biological variance, which was rather high, pools of samples of one and the same tumor type, i.e., with a same anatomical-pathological classification were carried out. Thus, 3 pools of 4-5 tumor samples were carried out for each of the groups; pTa LG, pT1 HG, pT2 HG and controls.

3. Affymetrix Microarrays

Although a platform of microarrays based on cDNA had been previously worked with, it was known from the literature that there were other commercial platforms based on oligonucleotides which would allow obtaining expression results of a higher number of genes. Finally, the decision was made to use the Affymetrix platform given that there was a large amount of data available in the public database for this platform, virtually all the references mentioned its high results quality and a new microarray (U133 plus 2.0) had just been launched on the market which allowed determining the gene expression of most human genes.

The Affymetrix microarrays were hybridized and scanned by a specialized company (Progenika) and the raw expression data (or cel files) were directly analyzed under the R statistical environment using the RMA (Robust Multi array Analysis) algorithm.

4. Affymetrix Microarray Analysis

Once the standardized expression data for each clone had been obtained, the decision was made to study how the different samples which had been selected clustered together by carrying out an unsupervised cluster (FIG. **4**). In the latter, it could be observed that all the controls were clustered together and clearly differentiated from the tumors, which indicated that there are many differentially expressed genes between tumors and controls (diagnostic genes). In addition, it is also observed that the 3 pools of infiltrating tumors (pT2_1, pT2_2 and pT2_3) and high-grade superficial tumors (pT1 HG_1, pT1 HG_2 and pT1 HG_3) are clustered together and differentiated from the 3 pools of low-grade superficial tumors (pT1 LG_1, pT1 LG_2, pT1 LG_3), which should allow locating marker genes of either pathway (prognostic genes).

As a ranking system for comparing the different groups and obtaining the best differentially expressed genes, the decision was made to use the ratio between the maximum intensity value of the group with the lowest mean and the minimum intensity value of the group with the highest mean, in a logarithmic scale. This measurement is equivalent to the minimum fold change which could be obtained by comparing any replica of a group against any replica of the other group. The end result obtained were literally thousands of genes with sufficiently significant expression differences between tumors and controls.

5. Validation of the Microarrays Results by Means of Quantitative Real-Time PCR

Once the genes were ordered from more to less differentially expressed, the decision was made to verify the results obtained with a completely independent and, according to the literature, much more accurate technique, quantitative real-time PCR (qRT-PCR). Ten of the most differentially expressed genes were selected to carry out this technical verification and their gene expression was quantified by means of qRT-PCR using exactly the same pools hybridized in the microarrays (in order to be able to compare the results of both techniques) and using the individual samples of each pool (in order to be able to study the replicability of the actual qRT-PCR technique) (FIG. **5**). A regression coefficient of 0.978 was obtained in the comparison between microarrays and qRT-PCR, which indicated a very good replicability between the 2 techniques. When the arithmetic means of the individual samples obtained by means of qRT-PCR and their expression in the pools by means of the same technique were compared, the regression coefficient was 0.995, which confirmed that the bibliographic data of the fact that the quantification by means of qRT-PCR has an excellent technical quality.

6. Conclusion of Example 2

Taking into account the results observed by means of the quantification of gene expression using two completely independent techniques in a small group of genes, it can be extrapolated that the expressions observed by means of microarrays seem to be sufficiently reliable for being used to define a robust group of candidate genes for a subsequent, more extensive and specific analysis.

In parallel, it was concluded that although the microarrays were suitable for quantifying gene expressions, qRT-PCR was still more accurate, in addition to allowing higher RNA degradation levels in the sample without this negatively affecting the end result.

Example 3

First Selection of Candidate Genes

The final objective of this study was to select a reduced group of genes related to bladder TCC and that diagnostic and prognostic tumor information were obtained upon quantifying their expression. To that end, it has been verified that two techniques can be used, DNA microarrays and quantitative real-time PCR. By means of the microarray technology, thousands of genes are tested in each experiment and a larger amount of RNA with a better quality is needed to conduct the

experiments than with the qRT-PCR methodology. Furthermore, the latter is a more accurate technology and the exact number of genes of interest can be quantified. Therefore, the decision was made to use the TaqMan Low Density Array (TLDA) technology, based on qRT-PCR, in the subsequent phases of the study.

1. Selection of 384 Genes for the TaqMan Low Density Arrays (TLDA) Cards

TLDA are microfluidic cards containing the lyophilized primers and TaqMan probe for a maximum of 384 genes (there are different TLDA configurations which allow analyzing from 384 genes in one and the same card and up to 48 genes and 8 samples in one and the same sample). Therefore, from the previously conducted experiments by means of Affymetrix microarrays hybridized with tissue RNA, a subgroup of 384 genes has to be selected. The most differentially expressed genes between tumors and controls (diagnostic genes) and also the differentially expressed genes between the three tumor groups: pTa LG, pT1 HG and pT2 HG (prognostic genes) were selected.

In addition, given that one of the objectives of the project was to work with bladder fluids, the intention in this phase of the project was to be able to study these 384 genes not with tissue RNA, as had been done up until now, but rather directly with bladder fluids (urine or bladder washings).

2. Collection and Processing of the Bladder Washings and Urine

The bladder washing samples were collected by barbotage intraoperatively, before the resection of the bladder tumor or before cystectomy. The urine samples were collected by spontaneous urination before the patient entered into surgery. Both the bladder washing samples and the urine sample were transported to the laboratory in ice immediately after being collected. The samples were mixed with ½5 volumes of 0.5M EDTA, pH 8.0 and were centrifuged at 1000×g for 10 minutes. The cell pellets were resuspended in 1 ml of TRIzol (Invitrogen, Calsbad, Calif., USA) and were frozen at −80° C. until the RNA extraction.

425 tumor bladder washing samples, 30 control bladder washing samples, 43 tumor urine samples and 158 control urine samples were collected and stored.

3. RNA Extraction and cDNA Synthesis

The RNAs were extracted according to the protocol of TRIzol (Invitrogen, Calsbad, Calif., USA) and were quantified by spectrophotometrically measuring the absorbance at 260 nm.

The cDNA was synthesized from 1 ug of RNA using the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, USA) according to the supplier's instructions, except that the final volume of the reaction was reduced to 50 μl.

4. Selection of "Taqman Gene Expression Products" and Quantitative Real-time RT-PCR (qRT-PCR).

Once the genes of interest were known, the primers and fluorescent probe (TaqMan Assays-on-Demand™ Gene Expression Products) were selected for the quantification of the gene expression by means of qRT-PCR in the Applied Biosystems web.

A microfluidic card (TaqMan Low Density Array, TLDA) was configured which contained 384 assays corresponding to diagnostic genes and prognostic genes and to endogenous control genes (FIG. **8**). This TLDA configuration allows analyzing a single sample per card. The table of FIG. **8** indicates the gene name and symbol, as well as the Affymetrix clone in which the differential expression of the gene was found. The name of the TaqMan Gene Expression Assay available from the Applied Biosystems selected for the TaqMan Low Density Array microfluidic card is also defined. This assay name is in turn indicating the gene region which will be amplified in the qRT-PCR. Finally one of the major transcripts which will be amplified with this assay (Ref Seq or Gene Bank mRNA) is indicated.

The PCRs were carried out in an ABI PRISM 9700 HD SDS (Applied Biosystems, Foster City, USA) according to the supplier's instructions.

A total of 60 samples were analyzed by means of 384-gene TLDA:

- 39 tumor bladder washing samples
- 15 control bladder washing samples
- 3 tumor urine samples
- 3 peripheral blood samples; this was carried out given that in the previous analysis, based on Affymetrix microarray, muscle tissue contamination had been observed in the supposedly pure bladder mucosa samples and there were signs for suspecting that in there could be contamination in the bladder fluid samples due to the immune system. Therefore, the decision was made to analyze 3 lymphocyte samples in order to be able to eliminate by comparison the genes which are highly expressed in blood (given that blood would be a constant contaminant in the bladder fluid samples from patients with bladder tumor).

5. Analysis of the 384-gene TLDA

Once all the PCRs were conducted, the threshold levels and baseline levels that were most suitable for each gene were established and the Ct (cycle threshold) or raw expression data by means of the SDS 2.1 program (Applied Biosystems).

Subsequently, the relative expression measurement of each gene or delta Ct (Ct of the target gene—Ct of the endogenous control, GUSB in this case) was calculated and it was studied how the individual samples clustered together by means of an unsupervised cluster (using Euclidean distances and UPGMA) (FIG. **6**). The first classification level which was observed in this cluster is the differentiation between the 3 samples from peripheral blood and the bladder fluid (bladder washings and urine) samples. In addition, the bladder fluids are sub-clustered into a group of samples which cluster together in the upper part of the cluster (from the B155-RV_T2high sample to the B288-RV1_TaG2high sample) and which is formed only by tumor bladder fluid samples, and another group of samples in the lower part of the cluster (from the B71-RV_TaG2lowCIS sample to the B109-RV_T2high sample) which is formed by a mixture of tumor and control bladder fluids. Inside the upper cluster, high-grade and low-grade tumors can in turn be distinguished, whereas in the lower cluster there is a clustering with almost only control samples and another clustering with a mixture of controls and tumors, It must be taken into account that a change has been made from analyzing tissue in pools to bladder fluids in individual samples, therefore this loss of discrimination power by means of a cluster was relatively predictable.

The objective of this analysis was to reduce the genes to be studied in the next phase, with a higher number of samples, from 384 to 96. Different parameters were taken into account for the process for selecting the best genes, including the previously described statistical parameter (minimum fold change), but also the logarithmic scale proportion of the medians of the 2 compared groups (median fold change) and an individualized manual analysis by genes of the different intensity values. This allowed reducing the initial group of 384 genes to the 96 genes required for the next phase of experiments (FIG. **9**).

Example 4

Second Selection of Genes to Increase the Diagnostic/Prognostic Power

In this phase of the work, the objective was to increase the discrimination power between tumor and control samples. To that end, the intention was to analyze a higher number of bladder fluid samples and reduce, if possible, by at least half the number of genes on which the initial prototype of this diagnosis and prognosis system should be based.

1. Samples to be Analyzed and 96-gene TLDA

The microfluidic cards (TaqMan Low Density Arrays) containing 96 assays (FIG. **9**) were configured and processed in the same manner as with those of 384 genes, with the difference that this TLDA configuration allows analyzing 4 samples per card.

A total of 80 samples were analyzed by means of 96 gene-TLDA:

42 tumor bladder washing samples
8 control bladder washing samples
15 tumor urine samples
15 control urine samples

2. Analysis of the 96-gene TLDA

Given that the technology used in the previous phase of experiments (Example 3) was exactly the same as in this example and the genes analyzed in this phase were already included in the previously analyzed 384-gene TLDA, the decision was made to extract and add the 60 samples of Example 3, with the data of the new 80 samples (Total=140 samples).

A first analysis was conducted by means of an unsupervised cluster of the 140 samples with the expressions of the 96 genes and 2 clearly distinguished large groups (FIG. **7**) could be observed. In the first group (FIG. **7**.B), all the samples are tumor samples without exception. In contrast, in the second group (FIG. **7**.A), most of the samples are controls, but there are some tumor sample, with a genetic profile that cannot be distinguished from normal sample, The conclusion which could be extracted from this result is that most tumors had characteristic genetic profiles that were differentiated from the control samples, although there were some cases in which the general profile was not distinguished from a normal sample and, therefore, they could not be detected. The same effect as for Example 3 was being observed, although the discrimination capacity in the samples was now higher.

Based on the data observed from the clusters and in proper exploratory analyses attempting to use other classification algorithms (such as discriminating linear analysis, k-nearest neighbor (KNN), etc.), it could be observed that the problem of the discrimination of some tumors in relation to control samples persisted. The new working hypothesis was that any system for the global calculation of a discriminating measurement using a specific group of genes had the same problem, This consisted of the fact that, due to the high heterogeneity of the tumors, it was relatively easy to recognize the profiles of most of them, which would have mostly similar alterations, although there would always be a minority of cases for which the global behavior of the genes selected for their analysis would not be distinguished from the control samples, because they would have altered minority pathways.

To detect both majority and minority tumors, an "alarm system" was established by means of establishing a range of values between which the control samples ranged and adding a confidence interval such that a point could be determined from which an expression that was higher (or lower in the case of underexpressed genes) would indicate a tumor, regardless of the expression values observed in the other genes. The advantage of this system is that, although the tumor has general expression profiles similar to healthy samples, if one of the alarm genes is triggered, it allows affirming that the sample is a tumor sample.

The first step in the development of said system was the estimation of the expression ranges of the controls and their confidence intervals. Since it was very important for the control values to not have technical errors which would falsely alter the ranges, the decision was made to eliminate the controls that did not have a minimum quality level. To calculate this quality measurement, 3 genes (GUSB, 18S and PPIA) were used, which were furthermore useful as endogenous controls (by calculating their geometric mean) for the relative quantification of all the genes. By analyzing the individual behavior of the distribution of each gene, it was not possible to verify that a sufficient fit to a normal distribution was met, therefore confidence intervals based on its variance could not be established. As an alternative, the decision was made to establish arbitrary and fixed confidence intervals with different stringency levels (the decision was made to use double, 4 times or 8 times the value of the control with expression values that were more similar to the tumors as a threshold point).

Once the threshold point for each gene was determined, all this information was summarized in a matrix with the 96 genes against the tumor samples. The values which did not exceed the threshold level were marked with 0 and those that did exceed it were marked with 1 (for every stringency level). To select the best genes (with which the intention was made to reduce the profile to at least 48), two properties were taken into account: 1) that the gene could detect a high number of tumors (searching for the one having a higher sum of values 1) and 2) that this detection were as independent as possible from other alarm genes (in order to be able to detect the maximum of minority pathways).

As a result, the number of interesting genes could have been reduced to less than 48, although for technical reasons and being conservative, the decision was made to maintain this number for their analysis in subsequent phases, because some intervals in the controls might not be completely correct (due to the low number of control samples analyzed up until now).

To automate the process for analyzing new samples from the genetic profile of the 48 selected genes (FIG. **10**), a computer program was created which, starting from the Cts results obtained from the qRT-PCR, can carry out a diagnostic prediction. This program can use different parameter files (depending on the stringency in the intervals), therefore the sensitivity (SN) and specificity (SP) values vary. Using the least stringent parameter file (the threshold point being double the control that is closest to the tumors), SN=100% and SP=100% was obtained. In the case of the second parameter file (the threshold point being 4 times the control that is closest to the tumors), SN=98.96% and SP=100% was obtained. In the last case (the threshold point being 8 times the worst control), SN=97.93% and SP=100% was obtained. It is important to indicate that these results have been obtained on the same samples used to generate the parameter files, therefore an overfitting is probably occurring which it would be necessary to estimate in subsequent experiments with new samples.

Example 5

Development of a Final Diagnosis Model

The objectives in this phase of the project were to test and improve the tumor prediction model as well as to reduce to a minimum the number of genes used to carry out the prediction.

For this phase, it was necessary to amplify much more the group of tumor and control samples. 440 new samples were analyzed by means microfluidic cards with 48 genes, which have been added to the data of Example 3 (60 samples) and of Example 4 (80 samples).

Once the minimum quality controls in the samples have been carried out, they were analyzed by means of the previously described qualitative alarm model. The result obtained (SN=0.81 and SP=0.81) was rather different from that obtained with the final model of Example 4, therefore the decision was made to attempt improving it, because it probably had much overtraining.

From the observation of the discretized frequency histograms of each of the genes, it could be observed how the tumor and control samples were distributed. Due to the fact of having greatly increased the sampling, the overlapping limit between the distributions has been considerably reduced. It could also be observed that, although in a very low frequency, some control cases had expression levels that were very similar to the tumors.

Although at a conceptual level, the developed qualitative alarm system was still considered a good approximation to the cell behavior of the gene expressions, the impossibility to quantify the importance of each of the genes represented a serious limitation to the predictive power thereof.

Based on the same alarm concept, the decision was made to attempt developing a quantitative model, which was possible by using Bayes' conditional probability theorem.

Since the number of analyzed samples is sufficiently high, the probabilities that, given an expression value, the sample is either a tumor or a control can be estimated from the expression frequencies observed.

One of the advantages of a model based on Bayes' theorem is that it can be independently applied to each sensor gene. The gene expression observed will modify the a priori probability of being a tumor, giving an a posteriori probability, which can be used again as an a priori probability for the next gene. In fact, independence between the different genes is being implicitly assumed.

The final number of samples on which it has been possible to apply the model was 308 tumors and 156 controls.

When this model was iteratively applied on the 48 genes, a significant improvement was obtained in the prediction power of the previous qualitative model (SN=0.86 and SP=0.92), although by studying the frequency histograms it could be observed that many genes seemed to not provide significant information to the final model. Therefore, the proposal was made to select the sub-group of genes sufficient and necessary to capture the maximum of diagnostic information of the samples.

There was no clear way of carrying out the selection of most interesting genes by using the quantitative model. The old qualitative model did allow selecting the most informative genes and, in turn, with a higher independence between them. The result of using the best genes detected with the qualitative model (CTSE, MAGEA3, CRH, SLC1A6, PPP1R14D, IGF2, C14orf78 and KLF9) over the new quantitative model showed an important improvement in the results (SN=0.89 and SP=0.96).

In any case, the decision was made to attempt other approximations. From the visual analysis of the frequency histograms of the 48 genes, the apparently most informative sub-group (ANXA10, CRH, IGF2, KRT20, MAGEA3, POSTN, SLC1A6 and TERT) and with histograms that were most varied between one another (expecting that this fact would indicate a higher independence between them) was selected. The result obtained also showed a significant improvement in relation to the analysis of the 48 genes (SN=0.90 and SP=0.96).

Finally, since both the sub-group of genes obtained by means of the qualitative model and the visually selected genes showed improvements in relation to the initial quantitative model, the decision was made to combine the genes of the 2 approximations (ANXA10, C14orf78, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6 and TERT). The result of the combined model was slightly better than any of them (SN=0.91 and SP=0.96).

Once the model was obtained, the decision was made to study if there was any common pattern both in the tumors and in the controls that were badly classified. In the case of the controls, a significant presence of samples with tumors in contact with the urinary system (mainly prostate, kidney and penis) could be detected. These types of samples probably have common expression patterns with bladder tumors, therefore they can confuse the prediction model. Therefore, the decision was made to eliminate from the control samples all the cases with tumors which might be in contact with the urinary system.

The number of control samples decreased from 156 to 126, there also being 308 tumor samples. An important improvement (SN=0.90 and SP=0.93) was observed by using the quantitative model with the 48 genes on this new population. In the case of the 8 most independent genes, SN=0.91 and SP=0.97 was obtained. In the sub-group with the most interesting histograms, SN=0.91 and SP=0.97 was obtained. Finally, in the combined sub-group of genes, SN=0.93 and SP=0.97 was obtained. It can generally be seen by calculating the data again with each previously selected sub-group that the power of the model had been increased by eliminating these types of controls.

As regards the study of the badly classified tumors, a significant increase in the number of cystectomies present in this group was detected. It is believed that the prior transurethral resection (TUR) which is frequency performed very close in time to radical surgery could be altering the molecular profile which was observed, since the tumor masses have been physically removed partially or completely from the epithelial walls of the bladder. Although in this study, cystectomy cases have not been eliminated since the data are not conclusive, it is recommended to not include these types of sample in the analysis of new populations.

Example 6

Development of a Final Prognosis Model

Although the most important concern was tumor prediction (diagnostic prediction), there was also an interest in classifying the different types of tumors (prognostic prediction), which is the main objective of this section. This classification could allow further personalizing the treatment in each case.

The tumor classification is currently based on the macroscopic and microscopic observation in the pathological anatomy laboratory. Their classification is decided by means of more or less standardized observations, based on the depth of the tumor and on the microscopic appearance of the cells. Recent molecular studies seem to indicate that there are actually two differential genetic profiles which mostly separate superficial type tumors and infiltrating tumors.

To carry out a prognosis classification model, the different groups of tumor groups must be correctly separated. The anatomical-pathological observations do not ensure the

match with the behavior at molecular level of the samples, therefore it did not seem a good idea to derive a prognostic model only from this classification. The use of a classification system by means of an unsupervised cluster (which mostly separated the samples into 2 large groups) was chosen, in addition to taking into account the anatomical-pathological (AP) grade.

As a group of valid superficial tumor samples, it was necessary for them to cluster together according to the cluster in the group corresponding to them and according to AP, they had to be low-grade Ta, T1 tumors and without associated carcinoma in situ (cis). The infiltrating tumors had to belong to the corresponding group of the cluster and according to AP they had to be high-grade T1, T2, T3 or T4 tumors and any tumor with the presence of CIS.

In the group of samples defined as superficial tumors, 129 of the 308 tumors were classified. In the group defined as infiltrating tumors, 100 of the 308 tumors were classified. Finally, 79 tumor samples either had discrepancies between their anatomical-pathological classification and their molecular profile or were not clearly defined within the two major groups of the cluster.

The methodology used to create a model which would discriminate between superficial and infiltrating tumors is exactly the same as that used in Example 5 to obtain a diagnostic model.

When Bayes' theorem was applied using the 48 genes, a good classification was obtained (SN=0.97 and SP=0.96).

It could be observed that the genes interesting for diagnosis coincided to a great extent with prognostic genes by analyzing the frequency histograms. However, there were some genes (MCM10 and ASAM) which were not suitable for diagnosis and were suitable for prognosis, therefore these two genes were added to the 12 previously selected genes. The resulting model with 14 genes proved to work almost perfectly (SN=0.99 and SP=1.00). Table 1 includes the 14 genes, indicating the gene symbol and the name of the TaqMan Gene Expression Assay selected for the TaqMan Low Density Array microfluidic card.

TABLE 1

Gene symbol	TaqMan Gene Expression Assay
ANXA10	Hs00200464 m1
C14orf78	Hs00746838 s1
CTSE	Hs00157213 m1
CRH	Hs00174941 m1
IGF2	Hs00171254 m1
KLF9	Hs00230918 m1
KRT20	Hs00300643 m1
MAGEA3	Hs00366532 m1
POSTN	Hs00170815 m1
PPP1R14D	Hs00214613 m1
SLC1A6	Hs00192604 m1
TERT	Hs00162669 m1
ASAM	Hs00293345 m1
MCM10	Hs00218560 m1

The invention claimed is:

1. A bladder cancer diagnosis and/or prognosis kit, consisting of a set of probes suitable for the detection and quantification of the expression pattern of the combination of ANXA10, C14orf78, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6, TERT, ASAM and MCM10 genes.

2. A bladder cancer diagnosis and/or prognosis kit, consisting of a set of probes suitable for the detection and quantification of the expression pattern of the combination of ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6, TERT and MCM10 genes.

3. A bladder cancer diagnosis kit, consisting of a set of probes suitable for the detection and quantification of the expression pattern of the combination of ANXA10, C14orf78, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6 and TERT genes.

4. A bladder cancer diagnosis kit, consisting of a set of probes suitable for the detection and quantification of the expression pattern of the combination of ANXA10, CTSE, CRH, IGF2, KRT20, MAGEA3, SLC1A6 and TERT genes.

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